Analysis of a murine Pink1 deficiency in serotonergic and dopaminergic neurons in respect of Parkinson´s disease

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Abstract

Parkinson's disease (PD) is one of the most common age-related movement disorders. The main pathological symptoms include a degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNC) and the occurrence of Lewy bodies. As a result, PD patients typically exhibit severe motor symptoms like bradykinesia, tremor and strong gait impairments. Subtle gait impairments are attributed to the "non-motor" symptoms in the pre-motor phase of PD. In addition, PD patients can develop other non-motor symptoms like cognitive and olfactory impairments much earlier than the actual appearance of motor symptoms. Two main forms of PD are described: the familial form, caused by inherited mutations, and the sporadic form, mainly caused by environmental factors and risk genes. Mutations in the human gene Phosphatase and tensin homolog-induced kinase 1 (*Pink1*) are believed to lead to a loss of function of its kinase domain and is recessively inherited. In order to analyse possible PD related effects of Pink1 deficiency, the murine gene *Pink1* was knocked out (KO) by deleting exon 2 & 3 (Pink1_del2/3). The phenotypes of this mouse line exhibiting complete loss of Pink1 function was described in a previous study.

These mice exhibited olfactory impairments and decreased serotonergic innervation of the olfactory bulb (OB). Likewise, Pink1_del2/3 mice showed gait impairments, although no neuronal degeneration in SNC was observed in aged mice. In order to understand the underlying mechanism of the Pink1 deficiency-related phenotypes and to uncover possible compensatory mechanisms, transgenic mice with a specific KO of *Pink1* in serotonergic or dopaminergic neurons were analysed at young and middle age (mid-aged). Mice underwent a behavioural screening of open field testing (OF) followed by acoustic startle reflex analysis/prepulse inhibition (ASR/PPI), CatWalk testing and olfactory analysis. In addition, dopaminergic neurons were quantified in the SNC and the content of dopamine and serotonin was also measured. Furthermore, the respective metabolites were analysed in different brain regions. Finally, serotonergic innervation in the OB was examined. Overall, young and mid-aged mutant mice of both mouse lines did not display highly significant impairments in the OF, ASR/PPI and olfactory testing. The CatWalk analysis revealed some PD-related parameters affected in mutant mice. The neurotransmitter content did not differ in the different brain regions nor did the number of dopaminergic neurons in the SNC or the serotonergic innervation

of the OB. In summary, a murine Pink1 deficiency in serotonergic or dopaminergic neurons did not lead to neuronal degeneration or biochemical changes. In addition, strong PD-related behavioural phenotypes were not observed. Nonetheless, both mouse lines exhibited subtle PD-related gait phenotypes. In conclusion, the serotonergic and dopaminergic system potentially plays a role in the development of PD-related non-motor symptoms upon Pink1 deficiency.

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Content

List of Figures

List of Tables

Abbreviations

3-MT	3-Methoxytyramine
5-HIAA	5-Hydroxyindole-3-acetic acid
5-HT	Serotonin
5-HT*HCl	Serotonin hydrochloride
5T4	Oncofetal trophoblast glycoprotein
6-OHDA	6-hydroxydopamine
a.u.	Arbitrary units
ABC	Avidin biotin complex
Acb	Nucleus accumbens
AFC	Automatic footprint classification
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ASR	Acoustic startle reflex
C. Elegans	*Caenorhabditis elegans*
CB	Cerebellum
cko	Conditional knockout
CN	Caudate nucleus
cort.	Cortex
CPG	Central pattern generator
ctrl.1	Control 1
ctrl.2	Control 2
CW	CatWalk
D1R	Dopamine receptor subtype 1
D2R	Dopamine receptor subtype 2
DA	Dopamine
DAB	3.3'-Diaminobenzidine
DAT	Dopamine transporter
dest.	Distilled
DHBA	3.4-Dihydroxybenzylamine

DJ-1	Parkinson disease protein 7
DOPAC	3.4-Dihydroxyphenylacetic acid
Drosophila	*Drosophila melanogaster*
E	Epinephrine
ECD	Electrochemical detector
EDTA	Ethylenediaminetetraaceticacid
ERT	Estrogen hormone-binding domain
FCS	Fetal calf serum
FELASA	Federation for Laboratory Animal Science Associations
fl_PINK1	Full-length PINK1
floxed	Flanked by two loxP sites
FP	Front paws
GABA	γ-Aminobutyric acid
GAK	Cyclin-G-associated kinase
GBA	Glucocerebrosidase
GMC	German Mouse Clinic
GPe	External segment of the globus pallidus
GPi	Internal segment of globus pallidus
HC	Hippocampus
HMGU	Helmholtz Zentrum München – Deutsches Forschungszentrum für Gesundheit und Umwelt
HP	Hind paws
HPLC	High performance liquid chromatograph
HRP	Horseradish peroxidase
HVA	Homovanillic acid
ID	Identification
IDG	Institute of Developmental Genetics
IMM	Inner mitochondrial membrane
IS	Internal standard
IVC	Individually ventilated
IVF	*In vitro* fertilization
KD	Knockdown
kidn.	Kidney
KO	Knockout
LB	Lewy bodies

LC	Locus coeruleus
L-DOPA	L-3.4-Dihydroxyphenylalanine
LED	Light-emitting diode
LF	Left front
LH	Left hind
LRRK2	Leucine-rich repeat kinase 2
MB	Midbrain
MEX	Institute Molecular EXposomics
mid-aged	middle aged mice
Mfn1/2	Mitofusin 1/2
MPI	Max Planck Institute
MPP	Mitochondrial processing peptidase
MPTP	1-Methyl-4-phenyl-1.2.3.6-tetrahydropyridine
mt	Mutant
MTS	Mitochondrial targeting sequence
n	Number
NA	Numerical aperture
NE	Norepinephrine
NM	Neuromelanin
NS	Null stimulus
OB	Olfactory bulb
OF	Open field
OMM	Outer mitochondrial membrane
PARK	Parkinson disease genes
PARL	Presenilin-associated rhomboid like protease
PBS	Phosphate-buffered saline
PBS-T	Phosphate-buffered saline with triton
PCR	Polymerase chain reaction
PD	Parkinson´s disease
PFA	Paraformaldehyde
Pink1	Phosphatase and tensin homolog - induced kinase 1
Pink1 x DAT	Pink1_CKO x Slc6a3_CreERT2_TG
Pink1 x Pet	Pink1_CKO x Pet1_Cre_TG
Pink1_del2/3	PINK1 deficient mice
PP	Prepulse

PPI	Prepulse inhibition
PPN	Pedunculopontine nucleus
Put	Putamen
RF	Right front
RH	Right hind
RN	Raphe nuclei
RNAi	RNA interference
rpm	Revolutions per minute
S-	Not habituated odorant
S.E.M	Standard error of mean
S+	Habituated odorant
SD	Standard deviation
SNC	Substantia nigra pars compacta
SNCA	α-Synuclein
SNR	Substantia nigra pars reticulate
SP	Stimulus with prepulse
SPF	Specific pathogen free
SS	Single stimulus
STN	Subthalamic nucleus
STR	Striatum
T	Tendency
TAE	Tris-acetate-EDTA
TH	Tyrosine hydroxylase
TMD	Transmembrane domain
VM	Ventral midbrain
VPS35	Vacuolar protein sorting 35
VTA	Ventral tegmental area
wt	wild type

Chapter 1

Introduction

1.1 Parkinson's disease

Parkinson´s disease (PD) is one of the most common neurodegenerative disorders, first described in ancient times (Manyam, 1990, Jankovic, 2008). In 1690 the Hungarian physician Ferenc Pápai Páriz reported the occurrence of the cardinal PD motor symptoms: bradykinesia/akinesia, tremor, rigidity, and postural instability. A detailed description of the same symptoms by the British physician James Parkinson followed over 120 years later (Bereczki, 2010, Parkinson, 2002). Originally, James Parkinson named the disease "paralysis agitants" in his published "An essay on the shaking palsy" (Parkinson, 2002). Later in 1888, the "paralysis agitans" was named "maladie de Parkinson" or "Parkinson´s disease" by the physician Jean-Martin Charcot (Charcot *et al.*, 1888). It became apparent that during the course of PD the pigmented cells in the substantia nigra pars compacta (SNC) – nowadays known as dopaminergic neurons – specifically succumb to cell death. Thus, the loss of dopaminergic neurons in the substantia nigra, together with the PD-specific occurrence of cellular inclusion bodies – the Lewy bodies (LB) – are regarded as being the pathological hallmarks of PD (Fahn, 2017, Goetz, 2011).

The estimated worldwide incidence of PD ranges between 5 to > 35 new cases per 100.000 individuals per year. The discrepancies are due to differences in the demographics of population-based studies and study methods. The incidence of PD is rare before the age of 50 years and increases up to 5 to 10-fold at an age of 60 to 90 years (Poewe *et al.*, 2017). The worldwide prevalence of PD is about 0.3% but increasing steadily with age up to > 3% in people at an age over 80 years, indicating that age is the greatest risk factor for developing PD (Poewe *et al.*, 2017). In addition, apart from age, the incidence and prevalence of PD depends on, amongst other factors, ethnicity, geographic location, sex and genetics (Pringsheim *et al.*, 2014, Picillo *et al.*, 2017). In the future, the number of patients suffering from PD will increase as the world population ages. Based on published prevalence studies, it is projected that

in Western Europe´s 5 most populous nations and the world´s 10 most populous nations, the number of PD patients over an age of 50 years will have more than doubled, that is from 2005 to 2030 from around 4 million to 9 million affected individuals. Although several surgical and pharmacological treatment options are available, a cure for PD is not yet available. This results in a heavy burden on patients, caregivers, families, health and social support system (Rodriguez-Blazquez *et al.*, 2015).

Current treatment of PD patients relies mostly on the use of pharmacologic agents, like L-3,4-dihydroxyphenylalanine (L-DOPA), in order to improve motor symptoms and quality of life (Pires *et al.*, 2015). Unfortunately, chronic treatment with L-DOPA can lead to involuntary movements called dyskinesia, and patients develop tolerance to the treatment (Lloyd *et al.*, 1975, Pires *et al.*, 2015). Hence, apart from pharmacotherapy, functional neurosurgery, gene therapy and cell-based therapies are the PD therapeutic approaches that are currently used in clinics, in clinical trials or still tested in preclinical research (Pires *et al.*, 2015). Still, all these therapeutic approaches aim at relieving the symptoms of PD and thus to rectify the loss of dopaminergic neurons in the SNC, which is part of the basal ganglia (Rice *et al.*, 2011). Importantly, these therapeutic options are not preventive, nor disease modifying nor do they take into account the multisystemic nature of the disease (see below). Thus in order to develop these preventive and disease modifying therapies, it is of highest importance to understand the aetiology of the disease in order to develop and discover new therapeutic avenues to slow down, halt or even prevent the development of the disease.

1.2 Symptoms and pathology of PD

Apart from the loss of dopaminergic neurons in SNC, another dominant pathological characteristic in PD is the occurrence of cytoplasmic inclusion bodies – LB (Spillantini *et al.*, 1997, Holdorff, 2006). Braak used the progressive occurrence of LB to define different stages of disease progression. He described how the disease progresses most likely in an upward direction starting from the dorsal motor nucleus of the nervus vagus in the brainstem and the olfactory bulb (stage I). Hence, PD pathology progresses over the pons (stage II) to the midbrain (stage III) and the basal prosencephalon and mesocortex (stage IV) and finally to the cortex and neocortex (stage V and VI) (Braak *et al.*, 2003a, Braak *et al.*, 2003b).

1.2.1 Motor symptoms of PD

Due to the loss of dopaminergic neurons, typical motor symptoms of PD occur. These are bradykinesia and akinesia that is slower movements or speed and decreased spontaneous movements respectively. A rhythmic involuntary movement of body parts, known as tremor, is also observed in PD patients. In addition, PD patients suffer from muscle rigidity, which results in an increased resistance to passive movements. All the aforementioned motor symptoms of PD are rather an early feature of clinically diagnosed PD, whereas postural instability with a flexed posture are dominant in late PD stages (Smith *et al.*, 2012).

Loss of dopaminergic neurons in the SNC results in dysfunctional basal ganglia circuitry – the underlying basis of the observed motor symptoms. The basal ganglia refers to a group of subcortical nuclei, which are engaged primarily in motor control among other functions like motor learning, behaviour and emotion (Lanciego *et al.*, 2012). In detail, the basal ganglia comprise – among others – the striatum to which the caudate nucleus (CN), putamen (Put) and the nucleus accumbens (Acb) refer (Lanciego *et al.*, 2012). All these three nuclei are considered as (1) input nuclei, which receive information mainly from the cortex, thalamus and substantia nigra. The basal ganglia (2) output nuclei consist of the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulate (SNR), which send basal ganglia information to the thalamus and mesencephalic motor regions, like the pedunculopontine nucleus (PPN). Neurons, located between the input and output neurons of the basal ganglia are defined as (3) intrinsic nuclei and comprise the external segment of the globus pallidus (GPe), the subthalamic nucleus (STN) and the previously mentioned SNC (Lanciego *et al.*, 2012, Poewe *et al.*, 2017). Dopaminergic neurons of the SNC innervate striatal neurons, which are subdivided into projection neurons (90%) and interneurons (10%). Projection neurons are also named medium-sized spiny neurons and express the dopamine receptor subtype 1 (D_1R) and subtype 2 (D_2R). Under healthy conditions, dopaminergic neurons of the SNC and cortical neurons activate striatal D_1R expressing neurons of the direct pathway. Subsequently, these neurons lead to an inhibition of the GPi and SNR, which inhibit the thalamus. Since the inhibition of the thalamus is inhibited, thalamic neurons lead to a cortical excitation (Figure 1.1). In turn, dopaminergic neurons of the SNC inhibit striatal D_2R expressing neurons of the indirect pathway, whereas cortical neurons activate striatal D_2R expressing neurons to a higher extent. Subsequently, these neurons lead to an inhibition of the

GPe, which inhibits the activation of GPi and SNR by STN neurons. Hence, thalamic input to the cortex is inhibited (Figure 1.1). In summary, the direct pathway leads to an enhancement of motor activity whereas the indirect pathway results in an inhibition of motor activity. Apart from the mentioned network within the basal ganglia, the basal ganglia receive additional inputs by interacting with the (sub)cortical projections in order to select and inhibit simultaneously occurring signals (Vogt Weisenhorn *et al.*, 2016, Lanciego *et al.*, 2012, Poewe *et al.*, 2017).

This simplified model of the basal ganglia circuitry is part of the explanation for the motor deficits described in PD patients. Since dopaminergic neurons in the SNC die, there is a reduction of dopaminergic nerve terminals in the striatum, which leads altered information processing within the direct and indirect pathway of the basal ganglia. As a result, the γ-Aminobutyric acid(GABA)ergic signalling of the output neurons is increased, followed in an increased inhibition of the motor output (Blandini *et al.*, 2000).

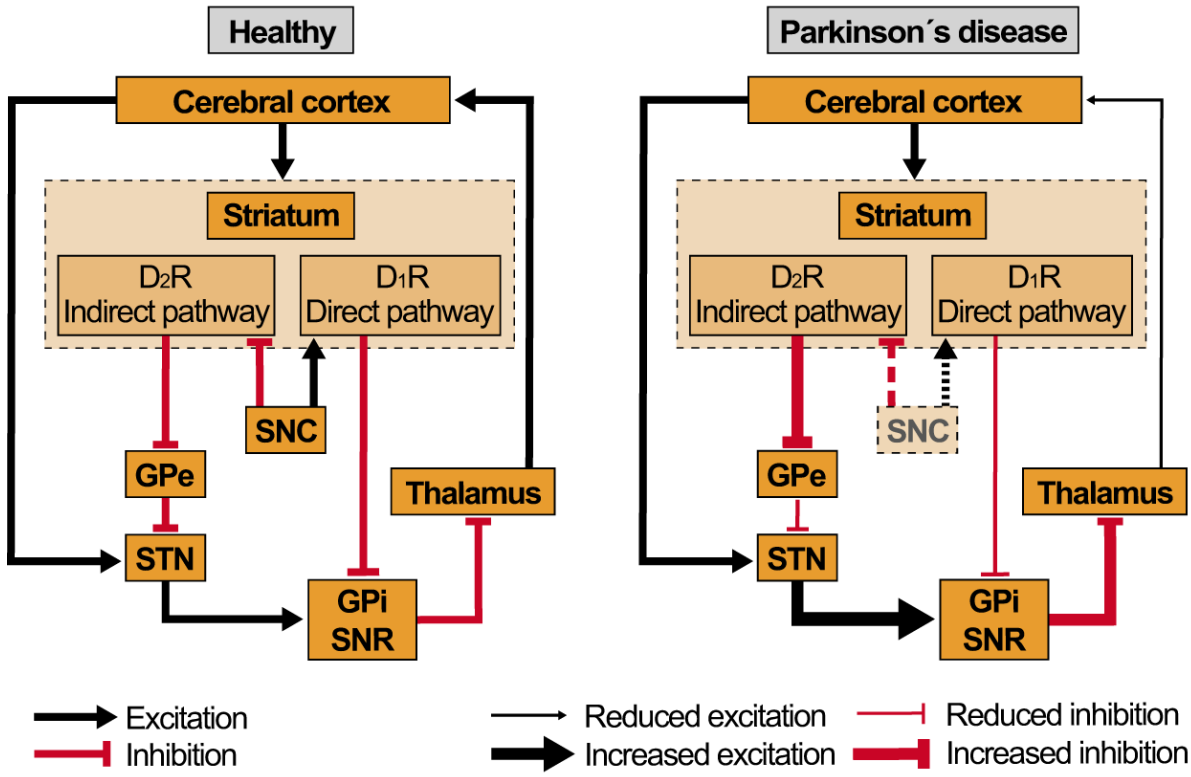

Figure 1.1: Simplified overview of basal ganglia motor circuit under healthy and PD conditions. Under healthy condition for the direct pathway, dopaminergic neurons of the SNC activate striatal D_1R expressing neurons, which results in an inhibited inhibition of the thalamus and a resulting excitation of cortical neurons and an enhancement of motor activities. For the indirect pathway, dopaminergic neurons of the SNC inhibit D_2R expressing neurons, which results in an inhibited inhibition of the STN and an excitation of inhibiting neurons in GPi and SNR. Under PD condition, direct and indirect pathways are altered, leading to an increased inhibition of the motor output.

1.2.2 Non-motor symptoms of PD

Previous studies showed that several non-motor symptoms likely precede the onset of motor impairments and characterize the prodromal phase of the disease, which can take up to 20 years. As non-motor symptoms are considered slight gait alterations, sleep problems, dysfunction in the autonomic system (constipation), sensory dysfunction, like olfactory problems, or dysfunction in the mood system, like anxiety and depression (Poewe *et al.*, 2017, Chaudhuri and Schapira, 2009, Wolters *et al.*, 2000, Dijkstra *et al.*, 2014). These “unspecific” non-motor symptoms might be due to an ongoing degeneration of the dopaminergic system, that can be, however, compensated for largely. Indeed, during the prodromal phase, motor symptoms become apparent when more than 50% of the dopaminergic neurons in the SNC are lost (Schapira *et al.*, 2017, Schwarz *et al.*, 2000). In addition, the multisystemic nature of the non-motor symptoms suggests the involvement of neuronal systems beyond the dopaminergic system. For example depression is observed in 35 - 40% of PD patients and causes a severe loss of the quality of life (Cummings, 1992). However, one of the highest risk factors for depression is a decreased neurotransmission of the neurotransmitter serotonin (5-HT) (Tan *et al.*, 2011). Alterations of 5-HT levels were also observed in PD patients including a loss of serotonergic neurons in the raphe nuclei of post mortem PD patient’s brain (Kish *et al.*, 2008, Paulus and Jellinger, 1991). In sum, even though the neurodegeneration of dopaminergic neurons dominates PD pathology, the degenerative process affects far more than the dopaminergic system. Hence, in further PD research it is important to consider other neurotransmitter systems beyond the dopaminergic system. Moreover, there is an urgent need for research into the prodromal phase in order to develop disease halting or modifying therapies, if not prevention, before the SNC is damaged beyond a point of no return.

1.3 Forms of PD

Research over the last decades revealed that to date PD could be classified into two main groups defined by their aetiology. On the one hand, there is the sporadic form of PD – mainly occurring in the aged population – on the other hand, PD occurs as a familial form for which mutations in distinct genes are responsible.

1.3.1 Sporadic (idiopathic) PD

The fact that PD is highly associated with age already points towards the multifactorial nature of disease aetiology. Indeed, age is the most important risk factor for developing PD (Bennett *et al.*, 1996). Nevertheless, further environmental factors contribute to the aetiology of the disease. For instance, a review about pesticides summarized and concluded that any pesticide causes a more than 50% increased chance of developing PD (Gunnarsson and Bodin, 2017). Stress as a risk factor for developing PD was discussed, since stress can lead to dysregulated levels of dopamine, which in turn may be neurotoxic (Hemmerle *et al.*, 2012, Metz, 2007, Smith *et al.*, 2002). In addition, stress accelerates PD motor symptoms, which was shown in PD rat models injected with 6-hydroxydopamine (6-OHDA) (Smith *et al.*, 2008). In a population-based study, an increased risk of PD was associated with diabetes mellitus (Yang *et al.*, 2017). Also the use of certain drugs like statins was associated with a higher risk of developing PD in a retrospective case-control study (Liu *et al.*, 2017). Furthermore, the composition of the gut flora represents a PD risk factor. The microbiome connects to the nervous system via the vagus nerve and forming the microbiota-brain axis. Supporting this hypothesis, α-synuclein overexpressing mice born in a germ-free environment, developed mild PD pathophysiology – a phenotype that was aggravated by providing oral bacterial metabolites to the mice (Tremlett *et al.*, 2017, Sampson *et al.*, 2016). In addition, vitamin D deficiency was associated with PD by analysing vitamin D levels in PD patients (Wang *et al.*, 2015). Conversely, certain factors decrease the risk of PD. As an example, in a Danish cohort study, a full truncal vagotomy was associated with a decreased risk of PD compared to control subjects followed up over more than 20 years (Svensson *et al.*, 2015). Analysis of several case-control and cohort studies of the consumption of tobacco were analysed with the upshot that an inverse association between cigarette smoking or also coffee drinking and the risk of PD was established (Li *et al.*, 2015, Hernan *et al.*, 2002).

1.3.2 Familial PD

Over the last 20 years, it also became evident that mutations in specific genes elicit certain forms of PD. About 5 to 10% of all PD patients suffer from a monogenetic form of PD, identified by linkage analysis (Klein and Westenberger, 2012). In these monogenetic forms of PD, highly penetrant rare mutations cause the disease in clustered families, resulting in the familial form of PD (Thomas and Beal, 2007).

The mutation in the gene *SNCA* (*PARK1/4*; encoding α-synuclein), causing early-onset autosomal-dominant PD, was discovered almost 20 years ago by Polymeropoulos and colleagues (Polymeropoulos *et al.*, 1996, Polymeropoulos *et al.*, 1997) and thus represents the first case of genetic PD. Over the last years, several pathogenic sequence variations were identified in *SNCA* (Polymeropoulos *et al.*, 1997, Kruger *et al.*, 1998, Zarranz *et al.*, 2004, Lesage *et al.*, 2013, Kiely *et al.*, 2013, Proukakis *et al.*, 2013). Mutations in *LRRK2* (*PARK8*; encoding leucine-rich repeat kinase 2, also known as dardarin) are the most frequent known cause of late-onset autosomal-dominant PD. A minimum of six highly penetrant pathogenic mutations in LRRK2 have been described so far (Lill, 2016, Healy *et al.*, 2008, Aasly *et al.*, 2010, Nuytemans *et al.*, 2008, Mata *et al.*, 2005, Khan *et al.*, 2005, Kachergus *et al.*, 2005, Lu *et al.*, 2005). An additional late-onset autosomal-dominant PD-causing mutation was described in the gene *VPS35* (encoding vacuolar protein sorting 35) (Zimprich *et al.*, 2011, Vilarino-Guell *et al.*, 2011).

Mutations in the gene *PARK2* (encoding Parkin) were firstly described as causing an early-onset autosomal-recessive PD (Kitada *et al.*, 1998). Over 100 mutations were identified spanning all exons of *PARK2*. Mutations in *PARK2* are the primary reason for autosomal-recessive PD (Abbas *et al.*, 1999, Hedrich *et al.*, 2004). Mutations in *DJ-1* (*PARK7*), causing an early-onset autosomal-recessive PD, are extremely rare (Hernandez *et al.*, 2016) but include about ten different reported mutations (Klein and Westenberger, 2012, Bonifati *et al.*, 2003). Another gene causing an early-onset autosomal-recessive form of PD is *Pink1* (*PARK6*, encoding phosphatase and tensin homolog (PTEN)-induced kinase 1) with a disease outbreak in the fourth decade of life exhibiting a slow disease progression (Al-Rumayyan *et al.*, 2017). More than 60 different *Pink1* mutations have been identified, affecting all eight exons with equal frequency.

1.4 Pink1

Pink1 is ubiquitously expressed throughout the human and rodent brain and peripheral tissue mostly in skeletal muscle, heart and testis (Taymans *et al.*, 2006, d'Amora *et al.*, 2011, Gandhi *et al.*, 2006, Oliveras-Salva *et al.*, 2011, Unoki and Nakamura, 2001, Blackinton *et al.*, 2007). Pink1 is highly expressed in brain regions containing serotonergic and dopaminergic neurons (Taymans *et al.*, 2006). There are two PINK1 isoforms in the human brain: (1) the human full-length PINK1 (fl_PINK1) protein with a

molecular weight of 63 kDa and (2) the cleaved PINK1 with a molecular weight of 52 kDa (Beilina *et al.*, 2005, Yamano and Youle, 2013). The human gene *Pink1* spans eight exons. Structurally Pink1 is composed of an N-terminal mitochondrial targeting sequence (MTS), followed by a transmembrane domain (TMD) and highly conserved serine-threonine kinase domain (Silvestri *et al.*, 2005, Zhou *et al.*, 2008). Indeed, two thirds of *Pink1* mutations cause a loss-of-function affecting the kinase domain (Valente *et al.*, 2004b, Valente *et al.*, 2004a, Klein and Westenberger, 2012). Pink1 exerts several cellular functions; the most important is involved in the control of mitochondrial biology.

In short, in healthy mitochondria, fl_Pink1 undergoes a continuous turnover at very low levels in cells with physiological polarized mitochondria. For this purpose, the cytosolic fl_Pink1 is transported into the mitochondria via the TOM complex (on the outer mitochondrial membrane, OMM) and TIM complex (on the inner mitochondrial membrane, IMM) (Jin *et al.*, 2010). Here, a mitochondrial processing peptidase (MPP), located in the matrix, cleaves off the MTS of the fl_Pink1. Afterwards the TMD is cleaved within the hydrophobic site by the inner mitochondrial membrane protease presenilin-associated rhomboid like protease (PARL) between the amino acids Ala103 and Phe104 (Greene *et al.*, 2012, Meissner *et al.*, 2011). The resulting 52 kDa Pink1 is released into the cytosol and degraded by the proteasome via the N-end-rule pathway (Yamano and Youle, 2013). In case of disrupted mitochondrial membrane potential, the cytosolic fl_Pink1 is directed to the OMM, where it undergoes dimerization and autophosphorylation in order to recruit Parkin to the OMM and to regulate mitophagy by interaction with each other (Okatsu *et al.*, 2012, Okatsu *et al.*, 2015, Yang *et al.*, 2006). Further main functions of Pink1 signalling include regulation of complex I activity, phosphorylation of proteins in the apoptotic pathway, regulation of mitochondrial trafficking or implication in inflammatory responses (Arena and Valente, 2017).

In sum, the molecular functions of Pink1 are manifold and thus mutations in this gene are likely to result in dysfunction of many systems. Indeed PINK1 function has already been implicated in liver function, tumorigenesis and diabetes (Arena and Valente, 2017). The discovery of the PD associated genes and their functional analysis in model systems revealed that – amongst others – impaired central cellular processes, such as mitochondrial function, autophagy and cytoskeletal integrity are at the centre stage of the aetiology of PD. Thus, it might not be surprising that Pink1 might exert different

functions in different neuronal populations, leading to distinct phenotypes, a notion that can only be addressed by conditional mutagenesis.

1.5 Mouse models of Pink1

Since loss-of-function mutations in *Pink1* genes lead to hereditary autosomal recessive PD, studies on Pink1 deficient animal models became of interest (Klein and Westenberger, 2012). Several Pink1 loss-of-function mouse models were developed in the past in order to recapitulate the pathological features presented in human patients and to contribute to the understanding of human PD (Oliveras-Salva *et al.*, 2011).

One of the first Pink1 mouse models was described in 2007 by Zhou and colleagues (Zhou *et al.*, 2007b). By using RNA interference (RNAi), they silenced *Pink1* gene expression in more than 95% in mouse brain. They could not observe any degeneration of dopaminergic neurons in the SNC nor a decrease of DA, or its metabolites 3.4 dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum at an age of 6 months. In addition, *Pink1* RNAi mice did not exhibit differences in time spent on a rotating rod, a test for motor function, specifically for balance. This indicates that *Pink1* knockout (KO) in mice does not result in neurodegeneration or motor impairments (Zhou *et al.*, 2007a).

Also in 2007, a *Pink1* KO mouse model was analysed by Kitada and colleagues exhibiting a deletion within the gene spanning exons 4 - 7 (Kitada *et al.*, 2007). Likewise, Kitada and colleagues could not discover an altered number of dopaminergic neurons in the *Pink1* KO mice at the age of 2 - 3 months and 8 - 9 months. The tissue content of DA was unchanged in the striatum in these *Pink1* KO mice, which correlates with the unchanged mRNA and protein levels as well as with the activity of the enzyme tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine biosynthesis (Daubner *et al.*, 2011). However, *Pink1* KO revealed a decrease in evoked DA release in striatal slices at an age of 2 - 3 months. This indicates an altered dopamine release in *Pink*1 KO mice (Kitada *et al.*, 2007).

In another study, these mice of Kitada and colleagues (Kitada *et al.*, 2007) were further analysed with respect to mitochondrial function (Gautier *et al.*, 2008). The aconitase activity in striatum decreased in mutant mice. There was no alteration in the ultrastructure and total number of mitochondria in the striatum of 3 - 4 months and 24

months old *Pink1* KO mice; however, there was a slight increase in the number of large mitochondria at both ages. Respiration was impaired in mitochondria isolated from the striatum but not from the cerebral cortex of 3 - 4 months old mutant mice. Interestingly, there were mitochondrial defects upon exposure to oxidative stress as well as in mitochondria isolated from the cerebral cortex of old mutant mice. This indicates that aging and cellular stress exacerbates mitochondrial dysfunction in *Pink1* KO mice. (Gautier *et al.*, 2008).

In another study these mice were exposed to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Pink1-deficient mice exhibited a significantly higher degeneration of dopaminergic neurons in the SNC and reduction of dopamine transporter (DAT) positive fibres in the striatum in comparison to wild type mice. The same effect was seen in mice with a shRNA-mediated knockdown (KD) of *Pink1*, which were also challenged with MPTP. Again, the *Pink1* KO and the shRNA-mediated KD itself did not cause neurodegeneration. Interestingly, viral-mediated expression of *DJ-1* and *Parkin* in the *Pink1* KO mice protected them from the loss of dopaminergic neurons in the SNC upon MPTP treatment. This indicates an induction of a possible compensatory mechanism upon murine Pink1 deficiency elicited by Parkin and DJ1 and/or that Pink1 acts upstream of Parkin and DJ-1 function (Haque *et al.*, 2012).

Similar results were obtained in another *Pink1* KO mouse line with a deletion of exons 2 - 5 (Wood-Kaczmar *et al.*, 2008). In this line mitochondrial stress was induced in dopaminergic neurons via overexpressing unfolded mitochondrial ornithine transcarbamylase under the TH promotor. This mitochondrial stress caused in wild types a significant reduction of dopaminergic neurons and of DA content in SNC. This pathology was accompanied by a significant reduction of rearing frequency. Interestingly, *Pink1* KO mice under mitochondrial stress exhibited a significantly higher neurodegeneration and reduced rearing frequency compared to single mitochondrial stressed transgenic mice. Thus, loss of Pink1 intensifies the mitochondrial and behavioural phenotype (Moisoi *et al.*, 2014).

In contrast to the effects of MPTP and mitochondrial stress, chronic exposure to low dose rotenone in *Pink1* KO mice from Kitada and colleagues (Kitada *et al.*, 2007) did not lead to a degeneration in SNC. Furthermore, mitochondrial integrity and ATP production were unaltered (Martella *et al.*, 2016). This indicates that low dose rotenone does not lead to PD-related phenotypes in Pink1 deficient mice and suggests that

strong challenges are needed to reveal a synergistic effect between environmental and genetic PD-causing factors.

Another study showed a higher loss of dopaminergic neurons in SNC upon α-synuclein overexpression in *Pink1* KO mice (deletion of exon 1) compared to Pink1 KD mice overexpressing α-synuclein (Oliveras-Salva *et al.*, 2014). This indicates that a *Pink1* KD is less vulnerable than a *Pink1* KO in mice.

In another *Pink1* mouse line the pathogenic G309D mutation, identified in human linkage studies, was inserted into the mouse gene resulting in loss of Pink1 protein (Gispert *et al.*, 2009). Thus, this mutation induced a loss-of-function, resulting in a *Pink1* KO. Mutant mice showed a significant reduction of body weight at an age of 12 and 16 months but not at an age of 4 months. Furthermore, mutant mice at 16 months showed significantly decreased locomotor activity in open field but no impairments in acoustic startle reflex. Even though dopaminergic neurons in SNC and nerve fibres in striatum were not degenerated in 18-month-old mutant mice, there was a significant reduction of striatal DA content at an age of 9 and 22 - 24 months. In addition, no LB in brainstem and SNC could be detected in aged mutant *Pink1* KO mice. With respect to mitochondrial function, mutant *Pink1* KO mice exhibited a significant progressive deficit of mitochondrial pre-protein import with age (3, 8 - 9 and 18 months) that correlated with deficits in mitochondrial respiration and thus ATP production. An increased mitochondrial fission and increased aggregation could, however, again only be observed under challenging conditions. (Gispert *et al.*, 2009). In sum, Gispert and collegues could uncover PD-related phenotypes due to a murine Pink1 deficiency.

In a study of Akundi and colleagues, they targeted exons 4 - 5 in order to generate a *Pink1* KO mouse line (Akundi *et al.*, 2011). As in the study of Gispert et al., mutant mice of this mouse line also displayed a significant reduction of DA content in striatum at an age of 6, 8.5 and 12 months. There was also increased turnover (DOPAC+HVA/DA) of striatal DA while the number of dopaminergic neurons did not differ in 12-month-old mice. In addition, Pink1 deficiency caused an aberrant expression of genes that regulate the innate immune response. Likewise, mitochondria from 2-month-old mutant mice showed a decreased calcium loading capacity.

Glasl et al. analysed *Pink1* KO mice (Pink1_del2/3) generated from a conditional mouse line in which exons 2 - 3 were floxed. In order to obtain a ubiquitous KO of *Pink1* these conditional mice were bred with the ROSA26 Cre deleter line (Glasl *et al.*, 2012). In this model, young and old mutant mice with an age of 3 and 24 - 26 months again

did not exhibit differences in open field and rotarod testing compared to wild type mice. However, for the first time a more sophisticated gait analysis was performed. Using the CatWalk system this analysis revealed gait parameters of the hind paws in 24 - 27 months old male Pink1_del2/3 mice. Specifically, the base of support, print length and phase dispersion were altered, which is indicative of PD-related gait impairments. In addition, for the first time mutant male mice were also examined for a further non-motor symptom of PD: olfactory impairment. This analysis revealed an impairment in discrimination of binary mixtures and olfactory sensitivity. Concerning cognitive ability, as revealed by the object recognition test, Pink1-deficient animals did not exhibit impairments. Again – like in the other models – there was no dopaminergic neurodegeneration even at old ages, supported by the fact that striatal DA tissue content was not altered in 6-month-old mutant mice, like the number of neurons in the SNC and locus coeruleus in 6 and 19 months old mice. However, these mice showed a specific decreased innervation of serotonergic neurons in the glomerular layer of the olfactory bulb (OB). Interestingly, the number of serotonergic neurons in the raphe nuclei were not altered. Concerning mitochondrial parameters, the mitochondria of these mice did not exhibit morphological changes. Nevertheless, and most interestingly, after an acute viral *Pink1* KD in primary cortical neurons of C57BL/6 wild type mice, a mitochondrial fragmentation was present three days after transduction. Interestingly, this effect was not observed five days after transduction. This specific experiment strongly hints towards compensatory mechanisms activated upon Pink1 deficiency, thus counteracting detrimental effects elicited by the loss of Pink1 function. In sum, the analysis of these mice revealed that Pink1 deficiency leads to non-motor and slight motor symptoms of PD, indicative of the pre-symptomatic (prodromal) phase of the disease. There is also evidence of compensatory mechanisms at play.

In summary, there are several different *Pink1* KD/KO mouse models. Yet, in the unchallenged state, no line exhibits degeneration of dopaminergic neurons in the SNC. Determination of striatal DA tissue content resulted in conflicting data. The observed changes were small so that the detection of these small changes depends on sample size, model used and possibly also on different genetic backgrounds. Furthermore, these slight changes indicate that even though these models do not show the pathological hallmark(s) of late PD they might increase sensitivity to additional stressors implicated in PD. Indeed, with exposure to environmental challenges like

mitochondrial stress or toxins, neuronal degeneration in SNC, including less striatal fibres, could be observed. Thus, these models support the multiple hit theory of the aetiology of PD (Sulzer, 2007). It indicates that PD is indeed a multifactorial disease not elicited by one single factor, such as a dysfunction of a PD associated gene. This single dysfunction in the animal model may lead to features akin to the prodromal phase of PD. This is supported by the observation that at least in one model it was shown that Pink1 deficiency induces behaviour resembling non-motor symptoms of the disease (Glasl *et al.*, 2012). Therefore, these animals are ideally suited to study this therapeutically important prodromal phase of the disease. They will be instrumental in identifying the neuronal systems implicated in disease aetiology, since, during the prodromal phase, the dysfunction of the dopaminergic system might not yet override the dysfunction in other neuronal systems.

Citation	Mutation	genetic background	Age	Challenge	Pathology					Behaviour*		Mitochondria		Others
					DA-system			other systems		Motor	Non-motor	morphology	function	
					no of TH	innervation of striatum	HPLC**	nr. of neurons	innervation					
Zhou et al., 2007	RNAi	FVB	6 monhts		→		→ DA, DOPAC, HVA			→ rotating rod				
Kitada et al., 2007	del exon 4 -7	129/Sv;C57BL/6	2 -3 months		→	↓	↓ DA							
			8 - 9 months		→		↓ DA							
Gautier 2008	del exon 4 -7	n.a.	3 -4 months									→ nr & ultrasturcture	→ cortex	
												↑ larger mito	↓ striatum	
			3 -4 months	H_2O_2/heat shock									↓ cortex	
			24 months									→ nr & ultrasturcture	↓ cortex	
												↑ larger mito		
Haque et al., 2012	del exon 4 -7	C57BL/6	n.a.		→	→								
				MPTP	↓	↓								
	del exon 4 -7			MPTP + ↑Parkin	→									
				MPTP + ↑DJ1	→									
	shRNA kd			MPTP	↓	↓								
					→	→								
Moisoi et al., 2014	del exon 2 - 5	n.a.	4,6,8,12 months	↑dOTC	↓					↓ rearing				
	wildtype		4,6,8,12 months	↑dOTC	↓		↓ DA			↓ rearing				
Martella et al., 2016	del exon 4 -7	n.a.	n.a.	low dose rotenone	→								→	→ ePhys SNC and striatum
Oliveras-Salva et al., 2014	shRNA kd	C57BL/6	n.a.		→					→				
	shRNA kd			↑ α-synuclein	→									
	del exon 1			↑ α-synuclein	↓									↑α-synuclein phospho.
Gispert et al., 2009	G309D	129/SvEv	4 months										↓	→ body weight
			9-10 months				↓ DA						↓	↓ body weight
			16 months							↓ spontan. Behaviour				↓ body weight,
			18 months		→								↓	
			22 months				↓ DA							
Akundi et al., 2011	del exon 4-5	129/Sv;C57BL/6	2 months										↓	
			6 months				↓ DA							
			8.5 months				↓ DA							
			12 months		→		↓ DA							
			n.a.	LPS										↑ cytokines, ↑ expression immuno
Glasl et al., 2012	del exon 2-3	C57BL/6	n.a.									→	→	
			3-6 months		→		→ DA	→ 5-HT	↓ 5-HT in OB	→ open field, rotarod				
			19-26 months		→					→ open field, rotarod	↓ olfaction ↓ gait			
			n.a.	acute Pink1 kd								↓ (compensated)		

Figure 1.2: Summary of published mouse lines implying a Pink1 deficiency. → not changed, ↑ up, ↓ down, TH = tyrosine hydroxylase, DA = dopamine, DOPAC = 3.4-Dihydroxyphenylacetic acid, HVA = Homovanillic acid, MPTP = 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine, LPS = Lipopolysaccharide, OB = olfactory bulb

1.6 Conclusion

Since PD is acknowledged as a multi-systemic disease, neurotransmitter systems beyond the dopaminergic system, such as the serotonergic or noradrenergic system are affected (Ohno *et al.*, 2015, Thobois *et al.*, 2017, Farrand *et al.*, 2017, Gong *et al.*, 2017). Indeed, serotonergic neurons of the raphe nuclei innervate the basal ganglia with the densest innervation in the substantia nigra (Parent *et al.*, 2011). Hence, a dysfunctional serotonergic system might play a role in the modulation of the dopaminergic output. However, the precise contribution of the serotonergic system to the development of PD-related symptoms is not known yet. To dissect and understand the role of different neuronal systems in PD symptoms, new approaches are needed. In the end, this would be useful knowledge in order to develop new therapies for PD. One such approach is the use of conditional mutagenesis in animal models of PD, which are concomitant to the full KO of the respective PD-related gene. Therefore, the focus of this project lies on the systemic analysis of conditional *Pink1* KO, either in the dopaminergic or in the serotonergic system.

In addition, PD is characterised by a disease outbreak at relatively late onset although PD-related genes are known to be expressed also during development (d'Amora *et al.*, 2011, Zechel *et al.*, 2010, Kuhn *et al.*, 2004). Thus, during development and in early adulthood compensatory mechanisms might be in place in order to deal with the effects of altered gene function. These still unidentified compensatory mechanisms might also be the underlying reason why most genetic mouse models do not exhibit overt PD pathology and PD symptoms (Antony *et al.*, 2011). However, additional stressors like MPTP, Manganese or 6-OHDA induce PD pathology and PD symptoms in PD animal models (Haque *et al.*, 2012, Langley *et al.*, 2017, Rial *et al.*, 2014). Indeed also challenged *Pink1* KO mouse models exhibited PD symptoms (Haque *et al.*, 2012, Moisoi *et al.*, 2014, Oliveras-Salva *et al.*, 2014). In addition, mitochondrial fragmentation – a PD-related phenotype – was shown with *in vitro* acute knockdown of *Pink1*. Interestingly, this phenotype was no longer present after several days, although the Pink 1 deficiency persisted (Glasl *et al.*, 2012). In sum, there are hints for compensatory mechanisms that counteract the gene deficiency possibly already during development and/or young adulthood. However, these mechanisms might decline with increasing age – possibly due to the decrease in adult neurogenesis, the decline of Wnt-signalling and neurotrophic signalling – whereat PD-related phenotypes are unmasked (Rao *et al.*, 2006, Berwick and Harvey, 2012, Parain *et al.*, 1999). Also,

as stated above, the disease outbreak of PD only occurs when already more than 50% of dopaminergic neurons are gone, proceeded by the neuronal degeneration in other brain regions (Schapira *et al.*, 2017, Chan-Palay, 1991, Schwarz *et al.*, 2000). Thus, there might not only be compensatory mechanisms at the molecular level as described above but it is highly likely that compensatory mechanisms also at the neuronal circuit level take place, which again might be already established during development.

Chapter 2

Aim of the study

In the last few years, several studies of Pink1-deficient mice have shown that Pink1 deficiency does not induce the full-blown neurodegenerative phenotype observed in Parkinson (PD) patients. However, slight alterations in the dopaminergic system have been associated with non-motor symptoms. Thus, these animals are veritable models of the prodromal phase of the disease and ideally suited to study this therapeutically important phase. In addition, the now available conditional mutagenesis has the potential to address two pressing questions in PD research:

1. What is the contribution of further neuronal systems to the observed PD-related phenotypes in the full knockout (KO) mice?
2. Are there compensatory mechanisms in place, hindering the outbreak of the disease until more than 50% of the dopaminergic neurons are lost?

The first question can be addressed by specifically inducing Pink1 deficiency in other neuronal systems. The second question can be addressed by inducing a Pink1 deficiency during adulthood so that compensatory mechanisms, possibly established during development, cannot prevent the occurrence of PD-related symptoms.

Thus, the aims of this thesis were the:

1. Analysis of mouse mutants in which Pink1 deficiency was specifically induced in the serotonergic system. This system was chosen because the KO of *Pink1* had an impact on the serotonergic innervation of the olfactory bulb and it is known that PD patients also exhibit a loss of serotonergic neurons.
2. Analysis of inducible mouse mutants in which Pink1 deficiency was elicited in the dopaminergic system. By using this model, it will be possible to determine (i) whether compensatory mechanisms impede the occurrence of strong PD-related phenotypes and (ii) to determine to which PD-related phenotypes the dopaminergic system contributes. The knowledge of this contribution is

important, since some of the PD symptoms are refractory to dopamine replacement therapy.

3. Performance of a longitudinal analysis of these mouse models in order to assess the impact of age on the phenotypes, since age represents the most important risk factor in the aetiology of PD.

The analyses were performed in a comprehensive manner, that is, at two time points after a standardized battery of behavioural tests proven to detect PD-related phenotypes. Mice were sacrificed and analysed for neurotransmitter content in the striatum, ventral midbrain and olfactory bulb. Additionally, morphological analyses were performed on the number of dopaminergic neurons in the substantia nigra pars compacta and serotonergic innervation of the olfactory bulb.

Chapter 3

Materials

3.1 Chemicals and working reagents

Table 3.1: Overview of the used chemicals and working reagents

Chemical	Company	Article
3.4 Dihydroxyphenylacetic acid (DOPAC)	Sigma Aldrich	102-32-9
3-Methoxytyramine hydrochloride (3-MT*HCl)	Sigma Aldrich	1477-68-5
5-Hydroxyindole-3-acetic acid (5-HIAA)	Sigma Aldrich	54-16-0
Acetonitrile	Sigma Aldrich	57-05-8
Agarose	Biozym	870055
Aqua-Poly/Mount	Polysciences, Inc.	18606-20
chocolate sprinkels	Back Family	Milchschokoladen Streusel, 22125668
Diaminobenzidine tetrahydrochloride hydrate (DAB)	Sigma	D5637-5G
di-Natriumhydrogenphosphat-Dihydrat (Na2PO4.2H2O)	VWR	28029.292
EDTA 0.5M fluid	AccuGENE®	51234
Epinephrine, Norepinephrine and Dopamine mix	Thermo Scientific	45-0206
Ethanol	Sigma	1.00014.2500
Ethidiumbromide	Sigma Aldrich	E1510

ethylenediaminetetraacetic (EDTA) solid	Sigma Aldrich	E5134-1kg
Fetal calf serum (FCS)	Thermo Scientific	26140079
GeneRuler™ 1 kb DNA Ladder + 6 x loading dye	Thermo Fisher	SM1333
GeneRuler™ 100 bp DNA Ladder + 6 x loading dye	Thermo Fisher	SM0323
Homovanillic acid (HVA)	Sigma Aldrich	306-08-1
Hydrogen chloride (HCl) 32%	Merck KGaA	Z0317213408
Hydrogen peroxide (H_2O_2) 30%	Sigma	H1009-100mL
Immersöl 518 F	Zeiss	444960
Internal standard (IS), 3.4-Dihydroxybenzylamine (DHBA)	Thermo Fisher	59-92-7
Isopropanol	Merck	113350
Miglyol	Caelo	3274
Mobile phase	Recipe	1210
Neg-50 frozen section medium, colorless	Thermo Scientific	6502
odorant [S-], Methyl trans-cinnamate	Sigma Aldrich	173282
odorant [S+], 2-Phenethyl acetate	Sigma Aldrich	290580
Paraformaldehyde (PFA)	Sigma	P6148-1KG
Peel-A-Way Disposable Embedding Molds	Polysciences	18646A
Perchloric acid solution (70%)	Sigma Aldrich	7601-90-3
Pertex	Medite GmbH	41-4012-00
Potassium chloride (KCl)	myneoLab	1710.10000

Potassium dihydrogen phosphate (KH_2PO_4)	AppliChem GmbH	A1043,1000
Proteinase K	AppliChem,	A3830,0500
Pursept-A Xpress	Schülke/ Merz Hygiene GmbH	
Serotonin hydrochloride (5-HT*HCl)	Sigma Aldrich	153-98-0
sodium acetat trihydrate	Sigma Aldrich	71188-5kg
Sodium azide (NaN_3)	Sigma	S2002
Sodium chloride (NaCl)	Merck Millipore	106404
Sodium hydroxide (NaOH)	VWR PROLABO	31624.290
Sodium hydroxide (NaOH) – pellets used for pH titration of PFA solution	Roth	9356.1
solvent, Diethylphthalat	Sigma Aldrich	W512206
Sterile Water	Biorad	1632091EDU
Sucrose	Sigma	S0389-5KG
Tamoxifen	Sigma Aldrich	T5648
Triton X-100	Bio-Rad, Cat.	161-0407
Trizma® base	Sigma	T1503-5KG,
Trizma® base	Sigma Aldrich	T1503-5kg
Xylol	Roth®	9713.3

3.2 Complete packages (“Kits”)

Table 3.2: Overview of the used complete packages (“Kits”)

Complete package	**Company**	**Article**
ABC Kit VECTASTAIN® Elite ABC KIT	Biozol	PK-6100
Wizard® Genomic DNA Purification Kit	Promega	A1125

3.3 Antibodies

Table 3.3: Overview of the used primary antibodies

Antibody	Company	Article
rabbit-anti-5-HT	Immunostar	20080
rabbit-anti-TH	Pel Freez®	P40101-0

Table 3.4: Overview of the used secondary antibodies

Antibody	Company	Article
Alexa Fluor® 594 donkey anti-rabbit	Mo Bi Tec	A21207
biotin-SP-conjugated AffiniPure Goat anti-rabbit	Dianova	111-065-003

3.4 Primer

Table 3.5: Overview of the used primer

primer	binding site	sequence (5´-3´)	Tm [°C]
neocass_for_AH	intron 1	GACAGTACTTGCCTAGCGTAG	61
ex2_rev_AH	Exon 2	CAGACACGCGCTTGGTTTTC	60
ko_rev_AH	intron 3	GAGCAATGCAGAAAGTCAGAGC	62
neocass_for	intron 1	GACAGTACTTGCCTAGCGTAGGTAG	67
neocass_rev	neo	TTGCTCAGCGGTGCTGTCCATCT	66
CRE-F	unknown	GATCGCTGCCAGGATATACG	60
CRE-R	unknown	AATCGCCATCTTCCAGCAG	57
Cre1_iDatcre	unknown	CTGCCAGGGACATGGCCAGG	67
Cre2_iDatcre	unknown	GGTCAAATCCACAAAGCCTGGCA	65

All primer were delivered from metabion international AG (Martiensried).

3.5 Solutions

Table 3.6 Overview of the prepared solutions

0.1% PBS - T	0.1% Triton X-100 (v/v) add PBS
0.2% PBS - T	0.2% Triton X-100 (v/v) add PBS
ABC solution	0.003% Avidin (v/v) 0.003% biotinylated peroxidase (v/v) add 0.1% PBS-T
DAB solution	1% DAB (w/v) add dest. H_2O
DAB working solution	5% DAB solution (v/v) 0.075 % H_2O_2 (v/v) add Tris-HCL
PBS	0.8% NaCl (w/v) 0.02% KCl (w/v) 0.18% $Na_2PO_4.2H_2O$ (w/v) 0.03% KH_2PO_4 (w/v) add dest. H_2O
PFA (4%), pH 7.4	4% PFA (w/v) add pre-warmed PBS pH adjusted with NaOH pellets & HCl
TAE buffer, pH 7.5	4.844% Trizma® base (w/v) 2.722% Na-acetat Trihydrat (w/v) 0.373% EDTA (w/v) add dest. H_2O
Tris-HCl, pH 7.5	12.11% Trizma® base (w/v) add dest. H_2O pH adjusted with HCl

3.6 Consumables

Table 3.7 Overview of the used consumables

Consumables	Company	Article
96 well PCR plates	Kisker	G060/H/1E/OA-SS
Cannula	Sterican	20 G, 0.9 x 40 mm
caps of 50 ml falcon tube Ø = 3 cm, h = 1 cm	Falcon®	734-0453
Cell strainer, 100 µm	Falcon®	352360
Clear snap cap	Supelco	50970
Coverslip	Roth®	H878
Cryotubes, 2ml	Greiner bio-one	122263
Falcons tubes, 50 ml	Sarstedt	62.547.254
Microscope slides	Thermo Fisher Scientific	J1800AMNZ
Nunclon ∆ Surface (24-well)	Thermo Fisher Scientific	142475
Nunclon ∆ Surface (6-well)	Thermo Fisher Scientific	140685
Pasteur pipette, PE-LD, 3.0 ml	Brand	747750
Pipette tips, TipOne®, 10/20 µl	Starlab	S1120-3810
Pipette tips, TipOne®, 1000 µl	Starlab	S1126-7810
Pipette tips, TipOne®, 20 µl	Starlab	S1120-1810
Pipette tips, TipOne®, 200 µl	Starlab	S1120-8810
safe-lock tubes 1.5 ml	Eppendorf	0030120086
safe-lock tubes 2.0 ml	Eppendorf	0030120094
shavings, Lignocel®	J. Rettenmaier & Söhne	S8-15
Snap ring vial	Supelco	29409-U
Transfer pipette, 3.5 ml	Sarstedt	86.1172.001

3.7 Instruments

Table 3.8 Overview of the used instruments

Instruments	Company	Article
10x Plan-Neofluar, NA 0.30	Zeiss	440331
20x Plan-Neofluar, NA 0.50	Zeiss	1004 - 989
40x EC Plan-Neofluar, NA 0.75	Zeiss	440350
5x Plan-Neofluar, NA 0.15	Zeiss	440320
Acoustic Startle Reflex Starter Package for Rat or Mouse Medium grid rod animal holder Sound attenuating cubicle Startle platform with load cell Startle platform attenuator	Med associates	MED-ASR_PRO1 ENV-264B ENV-022S PHM-250 PHM-255A
ActiMot System	TSE	
Ag/AgCl reference electrode	Thermo Fisher	044198
Axio Cam MRc	Zeiss	48-0026
Axioplan 2 imaging	Zeiss	201-0866
BioPette Plus 100 - 1,000 µl	Bioline	P3942-1000
BioPette Plus Multichannel Pipette 20 - 200 µl, 12 channel	Labnet international	LN P4812-300
cage barrier	own construction	19 cm x 15 cm 15.5 cm (l x w x l)
cage type II PC,	ANIMALAB	

252 mm x 167 mm x 140 mm (l x w x h) poly-carbonate, transparent		
CatWalk XT	Noldus	ZCWP-8001
Centrifuge	Thermo Fisher	Heraeus Multifuge 3SR+
centrifuge 5425	Eppendorf	5424000410
Comb for Gelsystem, 1.5 mm	Peqlab	50 inversions, 16 µl, Peqlab, 700-0528
Comb for Gelsystem, 1.5 mm	Peqlab	25 inversions, 40 µl, Peqlab, 700-0530 20 inversions, 20 µl, Peqlab, 700-0512 10 inversions, 46 µl, Peqlab, 700-0510
Cryostat	Thermo Fisher Scientific	HM560
Dividing wall for Gelsystem	Peqlab	700-0533
Gel documentation system	Herolab	E.A:S:Y Doc plus 440K
Glassy Carbon Electrode	Dionex	044113
Heating plate	IKA® Werke	RCT *basic*
Homogenizer	Bandelin Electronics	UW70, ultrasonic homogenizer
ice machine	Scotman	AF 30
incubator innova 4230	New Brunswick Scientific	8261-30-1008
Laborwaage 2 kg / 0.1 g	KERN	440-47N
Liquid nitrogen	Messer Cryosystems	Chromas 600KU
luxmeter, Mavolux	Gossen	5032C BASE
microwave oven	Sharp	R-937 IN

New: Column (C18, particle size: 4 µm, length: 150 mm, diameter: 4.6 mm)	Thermo Fisher	Accucore XL, 74104-253030
New: HPLC system Gradient pump, GP50 Autosampler, AS50 Detector, ED50A	Dionex	
Old: Column (C18, particle size: 3 µm, length: 150 mm, diameter: 4.6 mm)	Atlantis Columns	Atlantis T3, 0127321741
Old: HPLC system Gradient pump, GP40 Autosampler, AS40 Detector, ED40	Dionex	
Orbital shaker	Star Lab	SC3D001201
PCR machine	Eppendorf	MasterCycler Gradient
PerfectBlue™ Gelsystem Maxi L	Peqlab	700-0572
PerfectBlue™ Gelsystem Maxi S	Peqlab	700-0508
pH Level 1	inoLab	03280015
Pipetboy Easypet	Eppendorf	4430000018
Pipetman, 1 - 10 µl	Gilson	P10
Pipetman, 2 - 20 µl	Gilson	P20
Pipetman, 20 - 100 µl	Gilson	P100
Pipetman, 50 - 200 µl	Gilson	P200
Polymax 1040	Heidolph	543-42205-00-3
power supplies for electrophoresis	Pharmacia Biotech	EPS 200
precision scale	Sartorius	MC1 Laboratory LC 220 S
Security Guard Cartridges	Phenomenex	LB71270310
T-carrier		10 cm x 5 cm x 15.5 cm

		(l x w x h)
thermomixer comfort	Eppendorf	5355 000.011
Ultrasonic bath	Bandelin Sonorex	RK 510S
vortex-genie 2	Scientific Industries	SI-0236
water conditioning system	Millipore	Milli-Q biocel

3.8 Software

Table 3.9 Overview of the used software

Software	**Company**	**Article**
Stereo Investigator 11	mbf Bioscience	
Neurolucida	mbf Bioscience	
(old) HPLC, PeakNet 5.2	Thermo Fisher	
(new) HPLC, Chromeleon 6.5	Thermo Fisher	
ActiMot Software	TSE	
Advanced startle, SOF-828	Med associates	
CatWalk XT,10.5	Noldus	
EasyWin32	Herolab	
SPSS Statistics	IBM	
Graph Pad Prism	Graphpad Software, Inc	
Microsoft Excel	Microsoft Coorperation	
Microsoft Word	Microsoft Coorperation	
Adobe Illustrator	Adobe Systems	

3.9 Mouse lines

3.9.1 Ethic statement

All animals were handled and housed according to the approved federal guidelines for the use and care of laboratory animals (AZ 55.2-1-54-2532-144-10).

3.9.2 Pink1_CKO

The previous published paper (Glasl *et al.*, 2012) explains the generation of the conditional *Pink1* knockout (CKO) mouse line. In short, the exon 2 and exon 3 were

flanked by two loxP sites (floxed). In addition, a neomycin cassette was set upstream of the exon 2 and flanked by two FRT sites. The genotype of mice with a floxed exon 2 and exon 3 of the *Pink1* gene were defined as: Pink1:flox/flox when homozygous and Pink1:wt/flox when heterozygous. Mice obtaining the genotype Pink1:wt/wt did not exhibit any changes in the *Pink1* gene (Figure 3.1). Mice were bred with the wild type inbred mouse strain C57BL/6J of the Helmholtz Zentrum München (HMGU), Munich, Germany.

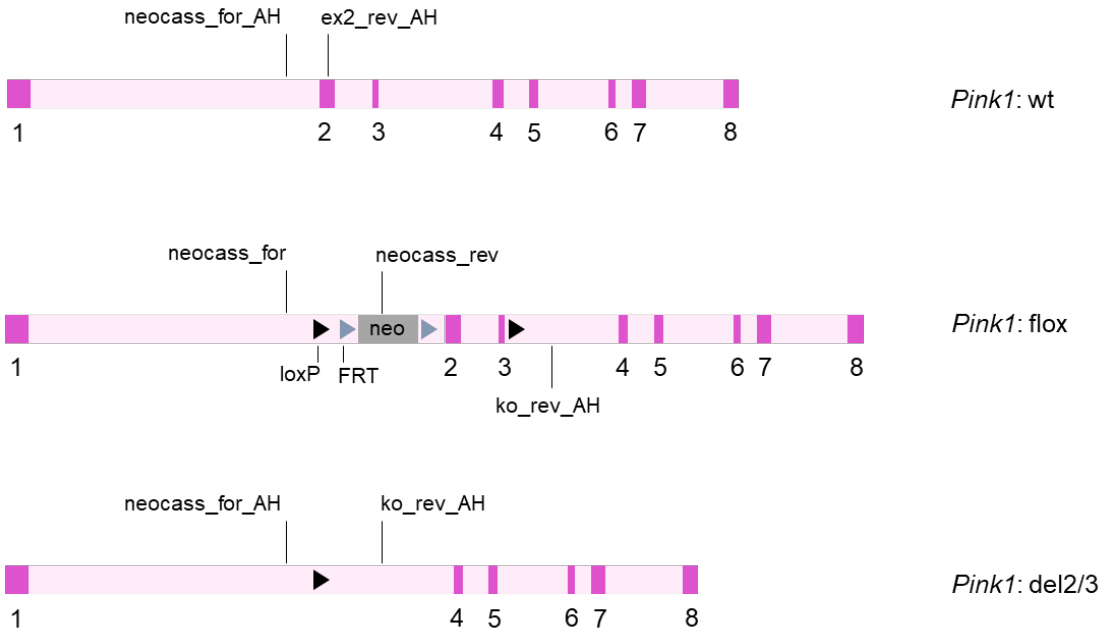

Figure 3.1: **Schematic overview of Pink1 in wild type, floxed and exon 2/3 deletion condition**. ***Pink1*: wt**, wild type *Pink1* is composed of 8 exons (purple). *Pink1*: flox, conditional knockout (CKO) of *Pink1* with flanked exons 2 and 3 by loxP sited. A neomycin cassette (neo, grey) is positioned upstream of the exon 2, flanked by FRT (blue) sites. ***Pink1*: del2/3**, after cutting of the active Cre recombinase on the loxP sites, the exons 2 and 3 and neomycin cassette are cut out, resulting in a *Pink1* knockout (KO). Primers used for genotyping were labelled (table 3.5 and table 4.1).

3.9.3 Pet1_Cre_TG

The previous published paper (Scott *et al.*, 2005) describes the generation of the mouse line Pet1_Cre_TG. In summary, the Cre recombinase was set under the control of the Pet1 promotor in order to be expressed in serotonergic neurons in the brain (Hendricks *et al.*, 2003). The genotype of mice expressing the Cre recombinase was defined as Cre:Cre+ in return the genotype of mice not expressing the Cre recombinase was defined as Cre:Cre-. Mice were obtained from Dr. Jan Deussing, Max Planck Institute of Psychiatry (MPI), Munich, Germany, who in turn received the

mice from Prof. Evan Deneris, Cave Western Reserve University, Cleveland, USA. The imported Pet1_Cre_TG mice were previously bred with inbred mice C57BL/6N of the MPI. Pet1_Cre_TG mice were transferred via in vitro fertilization (IVF) into the mouse facilities of the HMGU and bred with the wild type inbred mice C57BL/6J of the HMGU.

3.9.4 Slc6a3_CreERT2_TG

The previous published paper (Rieker *et al.*, 2011) describes the generation of the mouse line Slc6a3_CreERT2_TG (DATCreERT2). In summary, a cassette encoding for a tamoxifen induced form of the Cre recombinase (CreERT2, (Feil *et al.*, 2009)) was cloned in the ATG site of the murine *Slc6a3* gene, encoding for the dopamine transporter (DAT), which is expressed in dopaminergic neurons of the central and peripheral nervous system (Cerruti *et al.*, 1993, Ciliax *et al.*, 1999, Miller *et al.*, 1997). The genotype of mice expressing the Cre recombinase was defined as Cre:Cre+ in return the genotype of mice not expressing the Cre recombinase was defined as Cre:Cre-. Mice were obtained from Dr. Jan Deussing, MPI, who in turn received the mice from Prof. Günther Schütz, University of Heidelberg, Heidelberg, Germany. The imported Slc6a3_CreERT2_TG mice were previously bred with inbred mice C57BL/6N of the MPI. Slc6a3_CreERT2_TG mice were transferred via IVF into the mouse facilities of the HMGU and bred with the wild type inbred mice C57BL/6J of the HMGU.

3.9.5 Pink1_CKO x Pet1_Cre_TG (Pink1 x Pet)

The Pink1_CKO mice were bred with Pet1_Cre_TG mice (Figure 3.2). The recombinase-mediated recombination removed the exons 2 and 3 with the neo cassette, resulting in a knockout (KO) of *Pink1* in serotonergic neurons in the central nervous system, defined as mutant mice. The genotype of the mutant mice was defined as Cre:Cre+; Pink1:flox/flox. In comparison, mice expressing the Cre recombinase but possessing a wild type *Pink1* gene, were specified as control group 1 with the genotype Cre:Cre+; Pink1:wt/wt. Mice, expressing no Cre recombinase but owning the floxed exons 2 and 3 were called control group 2 with the genotype Cre:Cre-; Pink1:flox/flox. Both control groups were littermates of the mutant mice. Only mice with homozygous floxed or wild type *Pink1* gene were considered for the analysis. In contrast, Cre expressing mice (mutant and control 1) were always heterozygous for the Cre recombinase. After the IVF of the Pet1_Cre_TG mice, pups were already used for the

crossing with the Pink1_CKO mice (Figure 3.2). Since both mouse lines were bred on the different strains C57BL/6N (Pet1_Cre_TG) and C57BL/6J (Pink1_CKO), the resulting pups of the mouse line Pink1_CKO x Pet1_Cre_TG obtained a mixed genetic background of C57BL/6JN.

3.9.6 Pink1_CKO x Slc6a3_CreERT2_TG (Pink1 x DAT)

The Pink1_CKO mice were bred with Slc6a3_CreERT2_TG mice (Figure 3.2). The Cre recombinase CreERT2 was activated by injection of tamoxifen with an age of 10 weeks (Feil *et al.*, 2009). At five consecutive days, a daily volume of 100 µl of sterile filtered tamoxifen solution in miglyol with a concentration of 20 mg/ml were injected intraperitoneal into the mice with a 20 G cannula. All tested mice were injected with tamoxifen independent of the genotype.

After tamoxifen injection, the recombinase-mediated recombination removed the exons 2 and 3 with the neo cassette, resulting in a KO of *Pink1* in dopaminergic neurons in the central and peripheral nervous system, defined as mutant mice. The genotype of the mutant mice was defined as Cre:Cre+; Pink1:flox/flox. In comparison, mice expressing the Cre recombinase but possessing a wild type *Pink1* gene, were specified as control group 1 with the genotype Cre:Cre+; Pink1:wt/wt. Mice, expressing no Cre recombinase but owning floxed exons 2 and 3 were defined as control group 2 with the genotype Cre:Cre-; Pink1:flox/flox. Both control groups were littermates of the mutant mice. Only mice with homozygous floxed or wild type *Pink1* gene were considered for analysis. In contrast, Cre expressing mice (mutant and control 1) were always heterozygous for the Cre recombinase. After the IVF of the Slc6a3_CreERT2_TG mice, pups were bred on several generation with the wild type inbred mice of the mouse strain C57BL/6J of the HMGU, which led to a mixed genetic background C57NL/6JN. Since the Pink1_CKO mice obtained the genetic background of C57BL/6J, the following pups of the crossing Pink1_CKO x Slc6a3_CreERT2 (Figure 3.2) exhibited a mixed genetic background C57BL/6JN.

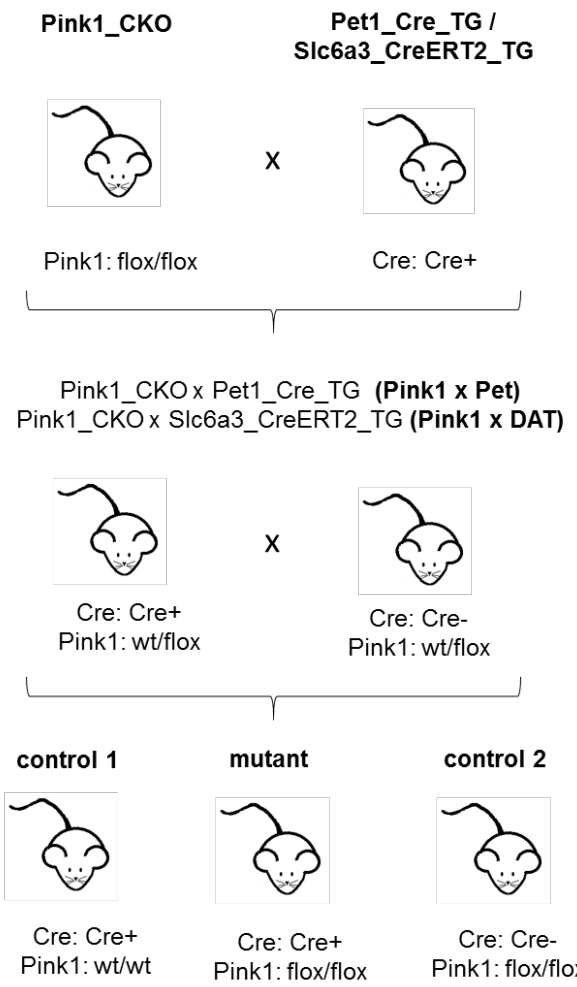

Figure 3.2: Breeding scheme of Pink1 x Pet and Pink1 x DAT mice. The homozygous floxed Pink1_CKO mice were bred with the heterozygous Cre positive Pet1_Cre_TG, respectively Slc6a3_CreERT2_TG mice. The pups, heterozygous for Pink1: wt/flox and for Cre: Cre+ respectively Cre: Cre-, were crossed in order to achieve the two control and mutant groups. The control groups were littermate controls.

Chapter 4

Methods

4.1 Mouse housing

All mice were born and housed in the mouse facility C-Streifen. For behavioural analysis, mice at an age of about 3.5 months were exported into the German Mouse Clinic (GMC) where all behavioural testing took place. Afterwards mice were housed for about 8 months until they were re-tested in the GMC. The hygiene status of the C-Streifen and GMC were kept constant via a specific pathogen free (SPF) barrier. The health monitoring was performed by following the Federation for Laboratory Animal Science Associations (FELASA) recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units (Nicklas *et al.*, 2002).

Males and females were housed separately. In order to avoid social stress, the arrangement of animals in cages did not change over the time. In the mouse facilities C-Streifen and GMC, animals were group housed with a maximum of five animals in type II long (Tecniplast Greenline, Sealsafe plus TM GM500) cages in individually ventilated caging (IVC) systems. The IVC cages operated at positive pressure where exhausts were removed via the connected ventilation system. The cages were changed by the animal caretakers on a weekly or fortnightly basis to fresh new IVC cages, depending on the amount of animals per cage and breeding. Hereby mice were transferred using forceps in a Class II AllerGard changing station (nuaire, NU-617-500E). All mice were served with fresh filtered water weekly. The temperature was kept between at 20 °C to 24 °C including a controlled humidity of 45 % to 65%. Mice were housed on a fixed 12 h light/dark cycle. Water (0.2 µm filtered) and food pellets (Altromin, 1314) were given ad libitum apart from the period of olfaction testing where a selected number of male mice were food restricted. The IVC cages were equipped with embedding material (Rettenmeier, Lignocel Select Fine) and cotton nestlet (AnimaLab).

4.2 Genoytping

4.2.1 DNA purification

In order to purify the genomic DNA from the tail clips, the Wizard® Genomic DNA Purification Kit was used. Per tail clip, a mixture of 120 µl EDTA solutions and 500 µl Nuclei Lysis solution was prepared and samples were chilled on ice. Tail clips with an optimal minimum length of 0.1 cm and 17.5 µl of Protinase K (20 mg/ml) were added to the EDTA/Nuclei Lysis solution and incubated over night at 55 °C. Afterwards, each sample was incubated for about 30 min at 37 °C with RNase solution and cooled down at room temperature for 5 min. Subsequent, 200 µl of protein precipitation solution were added followed by vortexing at high speed for 20 s and chilling on ice for 5 min. Thereafter, samples were centrifuged at 15000 x *g* at 4 °C and the supernatant was transferred into 600 µl isopropanol, gently mixed and centrifuged at 15000 x *g*. The supernatant was decanted and the DNA pellet was washed for two times with 70 % ethanol for 1 min at 15000 x *g*. The DNA pellet was air-dried, dissolved with 50 µl of H_2O and stored at 4 °C.

4.2.2 Polymerase chain reaction

In order to identify the genotypes of the mice regarding the *Pink1* gene and the presence of a construct expressing Cre recombinase, a combination of different primers (Table 3.4) were used for the polymerase chain reaction (PCR) (Figure 3.1).
To detect the wild type *Pink1* gene (Pink1 wt PCR), the primer neocass_for_AH, binding in the intron 1 and ex2_rev_AH, binding in the exon 2, were used, resulting in an amplified fragment of 781 bp. To detect the knockout of the exon 2 and exon 3 in the *Pink1* gene (Pink1 del2/3 PCR), the primer neocass_for_AH and ko_rev_AH, binding in the intron 3, were used, resulting in an amplified fragment of 583 bp. To detect the conditional status of the *Pink1* gene (Pink1 flox PCR) with loxP sites flanked exon 2 and exon 3 (floxed) and an upstream neo cassette, the primer neocass_for and neocass_rev, binding in the neo cassette, were used, resulting in an amplified fragment of 688 bp. To detect the construct, expressing for the Pet1_Cre recombinase, the primer CRE-F and CRE-R were used, resulting in an estimated amplified fragment of 600 bp since the binding side of the primer is unknown. To detect the construct, which encodes the Dat_Cre recombinase, the primer Cre1_iDatcre and Cre2_iDatcre were used, resulting in an estimated amplified fragment of 500 bp since the binding side of the primer is unknown.

The PCR mix (1x) was composed of 1.25 µl of the for_primer, 1.25 µl of the rev_primer, 10 µl of the master mix, 11.5 µl of H_2O with 1 µl DNA. The PCR programme is shown in table 4.1. The PCR reactions were pipetted in separate approaches. All PCR were performed for a minimum of two times per tail clip in order to avoid wrong interpretation of genotypes. In case of discrepancy of the genotypes, mice were re-genotyped or not considered for analysis.

Table 4.1: PCR programme

PCR	Temperature [°C]	time		primer	product
Pink1 wt	94	5 min		neocass_for_AH ex2_rev_AH	781 bp
	94	35 s	35 x		
	58.5	30 s			
	72	50 s			
	72	5 min			
Pink1 del2/3	94	5 min		neocasss_for_AH ko_rev_AH	583 bp
	94	35 s	35 x		
	58.5	30 s			
	72	50 s			
	72	5 min			
Pink1 flox	94	5 min		neocass_for neocass_rev	688 bp
	94	35 s	35 x		
	63	30 s			
	72	50 s			
	72	5 min			
Pet1 Cre	95	7 min		CRE-F CRE-R	ca. 600 bp
	95	30 s	35 x		
	63	1 min			
	72	1 min			
	72	7 min			
Dat Cre	95	5 min		Cre1_iDatcre Cre2_iDatcre	ca. 500 bp
	95	30 s	35 x		
	60	30 s			
	72	40 s			
	72	5 min			

4.2.3 Visualization of PCR fragments

For the detection of the amplified PCR products, a 2% agarose gel, containing ethidium bromide, was loaded with the PCR products and the DNA ladder. Before that, all PCR products were mixed with loading dye. If samples were filled on a big gel (PerfectBlue™ Gelsystem Maxi L), the gel was run for approximately 45 min at 150 mA. If samples were filled on a smaller gel (PerfectBlue™ Gelsystem Maxi S), the gel was run for approximately 30 min at 120 mA. Tris-acetate-Ethylenediamine-

tetraaceticacid (TAE) buffer was used as running buffer. Hence, for visualization of the PCR fragments, the agarose gel was put on a UV lightening plate and pictures were taken for documentation.

4.3 Preparation of mouse brains

In order to analyse the brain tissue, animals were sacrificed by cervical fractures, brains were removed and cut into two halves. Animals could not be perfused intracardially via pumping PBS and 4% paraformaldehyde (PFA) (w/v in PBS) into the blood system since one-half of the brain needed to be untreated to be used for neurotransmitter analysis. For this purpose, the brain regions olfactory bulb (OB), ventral midbrain (VM) and striatum (STR) were prepped, weighed and separately put into a cryotube and immediately shock frozen in liquid nitrogen and stored at -80 °C. In order to identify the exact weight of each prepped brain region, empty cryotubes were weighed before. The other halves of the brain were used for immunohistochemical analysis. The halves of the brain were separately transferred into falcon tubes filled with 50 ml of 4% PFA solution (w/v in PBS) and post-fixed for 48 hrs. Afterwards the brain halves were transferred into 20% sucrose solution (w/v in PBS) in which 0.001% of NaN_3 (v/v in sucrose solution) was diluted to protect tissue from contamination. Since mouse brains were not intracardially perfused, the half brains were more sensitive to cutting. Therefore, brains were not frozen and instead were stored at 4 °C.

4.4 Mouse line characterisation

In order to proof the selective KO of *Pink1* in the serotonergic and dopaminergic mice, different brain regions containing serotonergic and dopaminergic neurons, including control regions, were genotyped with respect to the gene *Pink1*.

4.4.1 Proof of selective Pink1 knockout in serotonergic neurons

Mice from the mouse line Pink1 x Pet were killed by cervical fracture, brains were taken out and immediately shock frozen on dry ice. Afterwards brains were cut into 200 µm thick layers with the cryostat and transferred on to microscope slides. With the help of a needle, the raphe nuclei were scratched out and transferred into the Nuclei Lysis solution with EDTA in order to purify the DNA (see 4.2.1). The transcription factor Pet1 is produced in serotonergic neurons in the raphe nuclei (RN) and not expected in the

cerebellum (CB) or cortex (cort.) (Hendricks *et al.*, 2003). In addition, Pink1 is synthesised in the RN, CB and cort. (Taymans *et al.*, 2006, d'Amora *et al.*, 2011). Therefore, the tissue RN, CB and cort. were genotyped to show the presence of the floxed *Pink1* and partially knocked out *Pink1*. For this purpose, the two PCR approaches were performed: Pink1 del2/3 PCR and Pink1 flox PCR as described in 4.2.2.

4.4.2 Proof of selective Pink1 knockout in dopaminergic neurons

Mice from the mouse line Pink1 x DAT were sacrificed and mouse tissue was prepped as described in 4.3. The dopamine transporter (DAT) is produced amongst others in the OB, SNC and VTA (Cerruti *et al.*, 1993, Ciliax *et al.*, 1999, Miller *et al.*, 1997) and less expected in the hippocampus (HC) and cortex (cort.). Peripherally, DAT was found among others in the kidney (kidn.). In addition, Pink1 is synthesised in the OB, HC, cort., MB and kidn. (Taymans *et al.*, 2006, d'Amora *et al.*, 2011). Therefore, the tissues OB, HC, cort., MB and kidn. were genotyped to show the presence of floxed *Pink1* and partially a knockout of *Pink1*. For this purpose, the two PCR approaches were performed: Pink1 del2/3 PCR and Pink1 flox PCR as described in 4.2.2. In addition, the mouse line Pink1 x DAT was tested for potential leakiness of the CreERT2 recombinase. Leakiness of CreERT2 is defined as a translocation of CreERT2 into the nucleus and recombination of the floxed DNA without previous injection of tamoxifen. Therefore, non-induced mice were also genotyped. Mutant mice and control group 2 mice were considered for the analysis.

4.5 Immunohistochemistry

In order to research the neuroanatomy of dopaminergic neurons in the SNC and serotonergic innervation into the OB, the prepped murine brains were cut, stained and analysed stereologically.

4.5.1 Cutting of mouse brains

Brains were washed in PBS solution to remove the 20% sucrose (w/v in PBS) solution with 0.001% of NaN3 (v/v in sucrose solution). Hence, brains were put in an embedding mold, embedded with section medium, and quick-frozen on dry ice. The brain halves were cut sagittal, respectively horizontal, into 40 µm thick serial sections of six using the cryostat. The cutting procedure was done at an object temperature of -18 °C to -

20 °C including a blade temperature of 1 °C below the object temperature. Brain sections were stored in 6-well plates filled with PBS solution with 0.001% of NaN3 (w/v in PBS) solution at 4 °C.

4.5.2 Staining of serotonergic innervation

Horizontal sections of the Pink1 x Pet mice and sagittal sections of the Pink1 x DAT mice were used for the staining of serotonergic innervation. In order to simplify the handling of brain slices, apart from the staining steps in antibody solutions, brain slices were transferred into cell strainers that fit in 6-well plates. Slices were washed two times with PBS for 5 min followed by two times with 0.2% PBS-T for 10 min. Hence, the slices were incubated in a 5% fetal calf serum (FCS) solution (v/v in 0.2 % PBS-T) for 2 h to block unspecific antigens. In order to detect serotonergic innervation, the neurotransmitter 5-HT (serotonin) was stained by incubating the slices in a 0.002 % primary antibody rabbit-anti-5-HT (Immunostar) solution (v/v in 0.2% PBS-T) over night at 4 °C. Staining steps were performed in 24-well plates with a lower volume of the used antibody solution and without using cell strainers. Afterwards, the unbound antibodies were removed by washing the slices three times for 5 min with 0.2% PBS-T. Then, slices were incubated for 45 min at room temperature in a 0.002% secondary antibody dilution (v/v in 0.2% PBS-T) with the dye Alexa594 labelled donkey anti-rabbit antibody (Mi Bo Tec). Subsequently, slices were washed three times with 0.2% PBS-T for 15 min, transferred on to microscope slides and mounted with Aqua Poly/Mount and cover slips.

4.5.3 Staining of dopaminergic neurons

Sagittal brain sections were used for staining dopaminergic cells. In order to simplify the handling of brain sections, apart from the staining steps with the antibodies, brain sections were transferred into cell strainers that fit in 6-well plates. Slices were washed three times with PBS for 10 min in order to remove the sucrose solution from the NaN_3. Afterwards slices were transferred in 0.3% H_2O_2 solution (v/v in PBS) for 10 min in order to destroy the endogenous peroxidases. After washing with 0.1% PBS-T twice for 10 min, the slices were incubated in a 2% FCS solution (v/v in 0.1% PBS-T) for 2 h to block unspecific antigens. In order to detect dopaminergic neurons, the enzyme tyrosine hydroxylase (TH) was stained by incubating the slices in a 0.0001% primary antibody rabbit-anti-TH (Pel-Freez) solution (v/v in 0.1% PBS-T) over night at 4 °C.

Staining steps were performed in 24-well plates with a lower volume of the appropriate antibody solution and without using cell strainers. Afterwards the unbound antibodies were removed by washing the slices three times for10 min with 0.1% PBS-T. Then, slices were incubated for 45 min at room temperature in a 0.003% secondary antibody dilution (v/v in 0.1% PBS-T) with the biotin-SP-conjugated goat-anti-rabbit antibody (Jackson ImmunoResearch). Subsequently, slices were washed three times with 0.1% PBS-T for 15 min and incubated with the avidin biotin complex (ABC) solution for 45 min, which was prepared 30 min prior to use. Slices were washed with PBS two times for 15 min followed by another washing step with Tris-HCL for 15 min. Finally, slices were incubated with the diaminobenzidine (DAB) working solution in the darkness since DAB is light sensitive. The incubation time was about 15 min; however, it depended on the reaction of the peroxidase with the H_2O_2. The resulting oxidation of the DAB caused a brown product. The reaction was stopped by washing the slices three times with PBS for 10 min. Thereafter, sections were transferred to microscope slides and dehydrated by incubation two times for 5 min in 70% Ethanol (v/v in dest. H_2O), followed by incubation two times for 5 min in 96% Ethanol (v/v in dest. H_2O). At the end, slices were incubated two times for 5 min in 100% Ethanol (v/v in dest. H_2O) and two times for 5 min in Xylol. After dehydration processes, brain slices were coverslipped with pertex.

4.6 Stereology

In order to analyse the stained murine slices, a stereological quantification was performed.

4.6.1 Quantification of dopaminergic neurons in substantia nigra pars compacta (SNC)

For the quantification of dopaminergic cells, the microscope Axioplan 2 imaging with the camera Axio Cam MRc was used. Brain slices were sorted in a serial manner and seven slices including the substantia nigra pars compacta (SNC) (Figure 4.1 A -G) were used. Since brain slices were cut in a series of six with a thickness of 40 µm, a layer of approximately 1.4 mm was considered for quantification with stereotaxic coordinates of approximately lateral 2.0 mm to 0.6 mm. For stereotactic quantification, the optical fractionator workflow of the software Stereo Investigator 11 (mfb Bioscience) was used. The principle was to scan spot wise selected regions of interest

in a random manner and to estimate a total number of cells in the marked region. Because of the dehydration process, slice thickness shrunk to a mounted thickness of 30 µm. The size of the counting frame was 100 µm x 100 µm and the sampling grid size was 200 µm x 200 µm. The top guard zone was selected at 3 µm and the dissector height at 20 µm. The region of interest, the SNC was surrounded with a 5x magnification lens and cells were counted at a 20x magnification lens. In case of a missing section, the Stereo Investigator software was able to calculate an estimated number. All counting was done in an unbiased manner without knowing the sex and genotype of the researched mice during counting process.

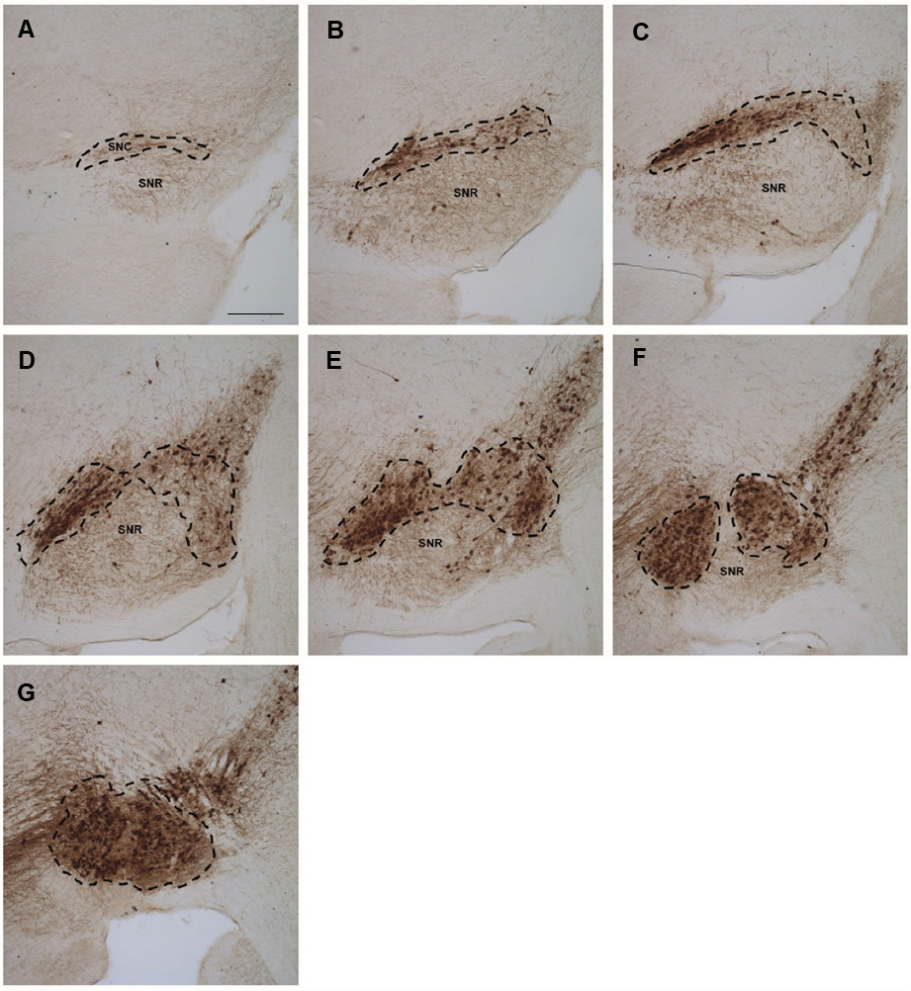

Figure 4.1: Serial overview of sagittal sections including substantia nigra pars compacta. (A - G) Selected slices used for quantification of TH+ cells in a serial manner of six. The dashed lines depict the bordering around the substantia nigra pars compacta (SNC) containing positive labelled cells for TH indicating the dopaminergic neurons. The neighbouring region, substantia reticularis (SR) exhibit very sparely dopaminergic neurons. Sections are shown from lateral stereotaxic coordinates of approximately 1.80 mm (A) to 0.72 mm (G).

4.6.2 Quantification of serotonergic fibres into the olfactory bulb (OB)

For quantification of the serotonergic fibre density in the glomerular layer of the olfactory bulb, the microscope Axioplan 2 imaging with the camera Axio Cam MRc was used. The sections were chosen with stereotaxic coordinates of approximately Bregma - 4.28 mm. With the help of the Stereo Investigator 11 (mfb Bioscience), one slice per mouse was analysed by putting a regular grid with a grid size of 60 µm x 60 µm over the glomerular layer of the olfactory bulb and all fibres crossing the grid were counted by a magnification of 40x. A random area in the glomerular layer of the OB with a size of 0.12 - 0.18 mm^2 in the Pink1 x Pet mice and 0.06 - 0.13 mm^2 of the Pink1 x DAT mice was chosen. For quantification of the fibre numbers, the software Neurolucida (mfb Bioscience) was used. All counting was done in an unbiased manner without knowing the sex and genotype of the researched mice during the counting process.

4.7 Analysis of neurotransmitter content

In order to analyse the concentration of the neurotransmitter content in the brain regions: OB, VM and STR, samples were analysed with high performance liquid chromatography (HPLC) system coupled with the electrochemical detection (ECD). The catechol structures of the analytics were oxidised to quinone, which produced a current. The measured current was directly proportional to the concentration of the analysed analytics.

4.7.1 Brain sample purification

Brain regions of the 4 month old animals were firstly purified, starting with the Pink1 x Pet mice followed by the Pink1 x DAT mice. In addition, samples of the same brain region were purified on a same time point having always a random mixture of groups. Thereby the effect of degradation of neurotransmitters while stored at -80 °C was minimised within brain samples, mouse lines and age groups. For the sample preparation, 200 µl of cold perchloric acid solution were added to the frozen brain samples in order to keep the analytics stable and precipitated proteins. In addition, 4 µl of cold 3.4-dihydroxybenzylamine (DHBA) was added as internal quantification standard (IS). The brain samples in mixture were thawed on ice. Afterwards, samples were lysed by 30 seconds of ultrasonication on ice with a UW70 ultrasonic homogeniser for 30 s. The homogenates were centrifuged at 7879 x *g* for 10 min at 4 °C. Subsequent, 40 µl respectively 20 µl of the supernatants were transferred in a

sample vials and closed with clear snap cap, which were suitable for injection into the HPLC system. The rest of the supernatant was stored at -80 °C.

4.7.2 HPLC measurements

Preparation of brain samples led to a loss of analytes. Knowing the IS and the analytes have the same properties, it was assumed that the loss of analytes and IS was equal during sample preparation process, defined as recovery [%]. Deductive, the component in the IS and in the analytes in the brain tissue exhibited the same recovery [%]. Thereby it was possible to calculate the original amount of analytics in the brain tissue before sample preparation since the concentration of the IS was known. The HPLC system was calibrated daily before the measurement of the neurotransmitter content took place by using a one-point calibration with a linearity of the calibration curve of $R^2 > 0.99$. The calibration solution contained the components dopamine (DA), 3.4-Dihydroxyphenylacetic acid (DOPAC), epinephrine (E), homovanillic acid (HVA), norepinephrine (NE), 3-Methoxytyramine hydrochloride (3-MT*HCl), 5-Hydroxyindole-3-acetic acid (5-HIAA), serotonin hydrochloride (5-HT*HCl), and 3.4-Dihydroxybenzylamine (DHBA) serving as the IS. All components were mixed to a final concentration of 1 ng/µl for each component (w/v, respectively v/v in Perchloric acid solution). Since the concentrations of the individual compounds in the calibration solution were known, the concentration of the corresponding analytics in the brain tissue were set into ratio and calculated.

The brain tissues of the 4 months old Pink1 x Pet and Pink1 x DAT mice were analysed with the HPLC system that consisted of the gradient pump GP40, the autosampler AS40, and the electro-chemical detector ED40. The injection volume of each sample into the HPLC system (old) was 40 µl. The isocratic mobile phase was diluted with 6% acetronitrile (v/v) and exhibited a flow rate of 0.5 ml/min through the Atlantis T3 column. The compartments of the column and the detector were heated to 30 °C. The software PeakNet 5.2 was used to control the system and to process the chromatograms.

The brain tissues of the 12 months old Pink1 x Pet and Pink1 x DAT mice were analysed with the HPLC system that consisted of the gradient pump GP50, the autosampler AS50, and the electro-chemical detector ED50A in which a volume of each sample of 20 µl was injected. The isocratic mobile phase was diluted with 0.25% acetronitrile (v/v) and exhibited a flow rate of 1 ml/min through the Accucore column.

The column oven was set to 40 °C. The software Chromeleon 6.5 was used to control the system and to process the chromatograms.
Prior to usage, the mobile phase was degassed in an ultrasonic bath. In the old and new HPLC system, a security guard cartridge in the same material according to the column was preceded to the column in order to prevent blocking and contamination of the column. All samples were injected once. The potential of the detector was set to 0.7 V against an Ag/AgCl reference electrode.

4.8 Behaviour analysis

In order to analyse possible non-motor and motor phenotypes, mice went through a standardised behavioural testing battery in the GMC.
Independent of the behavioural test, mice were tested in a blind random manner, apart from the knowledge of the sex. Males were always tested before females. About 30 minutes before behavioural testing, animals were transported from the housing room into the testing room to habituate. Animals were always tested separately and the body weight was recorded after testing. After finishing the testing of each mouse, the behavioural setup was cleaned with the disinfectant Pursept-A Xpress (Schülke). The behavioural testing started the earliest at 7 am and finished the latest at 5 pm, from Monday to Friday. The testing of CatWalk and olfaction were interrupted during the weekend.

4.8.1 Open field testing (OF)

In order to analyse the anxiety, locomotion and exploration, mice were tested in the open field. For this purpose, mice were placed in an open field area to record the movement of the mice by infrared sensors (Walsh and Cummins, 1976, Glasl *et al.*, 2012, Gates *et al.*, 2011). Hereby the ActiMot System was used.
The natural tendency to mice is to explore a novel environment. However, mice avoid open, unknown and potentially dangerous areas, which is represented by the centre of the open field (Choleris *et al.*, 2001). Thereby, the open field test is used to test the anxiety-related behaviour by alterations in exploration. However, with increasing time and exploration, animals are more familiar with the environment and the centre becomes less stressful (Holter *et al.*, 2015). Motor and/or balance problems can be indicated by reduced spontaneous rearing activity. Reduction in distance travelled or

speed movements can be also an indicator of motor problems but it could also reflect other changes in motivation or sensory modalities (Hölter and Glasl, 2012).

Up to five parallel open field tests were performed since there were five ActiMot Systems available in the GMC. Each ActiMot System was individually enclosed in a small, sound-attenuated room with an area of one square meter and white walls. The light of each room was set to 200 Lux in the centre of the open field area as varying light intensities influence the activity of the mice (Trullas and Skolnick, 1993). Since open field testing results changes over the timing of the day, all animals were tested before noon when the light cycle started (Hollaway, 2007).

The mice were placed into the open field periphery by putting the face of the mice towards the wall of the open field area. During the 20 min testing time, the spontaneous behaviour of the mice was recorded by the infrared sensors. The horizontal and vertical locomotor activity of the mice were monitored to give information about rearing frequency, distance travelled, location and the time course.

The ActiMot System consisted of a square-shaped frame with a lateral length of 48 cm (Figure 4.2). The frame was composed of two pairs of light-beam strips with each pair consisting of one transmitter strip and once receiver strip. Each strip was composed of 16 infrared light beam breaks with a distance of 28 mm. These basic light barrier strips were arranged at right angles to each other to determine the X and Y coordinates of the animals and following their location. The testing area consisted a 45 cm square with a coloured light grey smooth floor. The periphery zone was defined by an eight cm thick border. Hence, the centre of the testing area possessed 41.5% of the total surface. The testing area was surrounded by a transparent acrylic 39.5 cm high wall. Thereby, two additional further pairs of light-beam strips were set in front of each other and above the light-beam strips of the XY axis to measure the Z coordinates. Thus, rearing behaviour could be also measured. The centre of gravity of the mouse was calculated by the numbers of interrupted light beams on an XY axis. The activity settings were set up to record movement at a speed of > 0 cm/s. The rearing frequency was measured at a minimum duration of rearing of 200 msec.

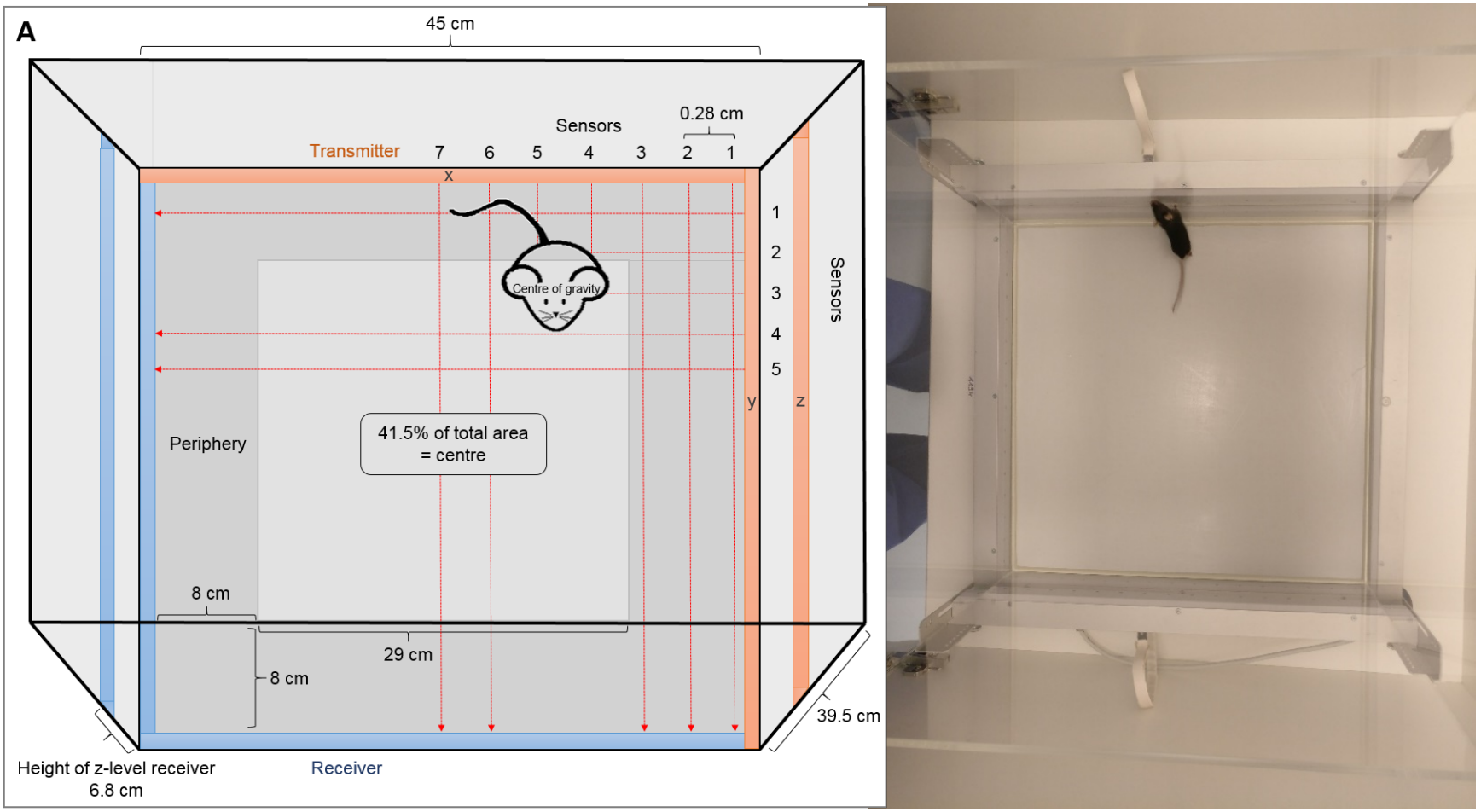

Figure 4.2: Experimental setup of the open field testing. (A) Schematic drawing of the open field testing area. The length of the squared open field testing is 45 cm with an 8 cm thick border, indicating the periphery. The inner centre was defined by a square with the length of 29 cm, resulting in 41.5% of the total testing area. To measure the movement of the mouse on the XY- axis, two pairs of infrared light beam sensors are installed in a rectangular angle. Each sensor pair is consisted of a transmitter (**orange beam**) and a receiver (**blue beam**). The height of the testing box conducts 39.5 cm. An additional pair of infrared light beam is installed on the wall at the height of 6.8 cm of the testing box to measure the movement of the mice on the Z-axis, interpreted as rearing. Each sensor consists 16 infrared light beams with a distance of 28 mm to each other. Whenever an even number of infrared light beams **(red dashed lines)** is interrupted, the centre of gravity is calculated to lie between the adjacent sensors. **(B)** Birds eye view of the open field testing box with a rearing black mouse. The picture was taken by the technician Jan Einicke from Institute of Developmental Genetics at the Helmholtz Zentrum München.

4.8.2 Acoustic startle reflex / prepulse inhibition testing (ASR/PPI)

In order to test the sensorimotor and hearing function, mice underwent an acoustic startle reflex (ASR) and prepulse inhibition (PPI) testing. The ASR measures the acute skeletal contraction due to an abrupt intensive acoustic stimulus. The most common form of modified ASR is the prepulse inhibition (PPI) testing. A short and less intensive sound is presented prior the ASR causing stimulus. As a result the ASR is reduced due to the prepulse (Lauer *et al.*, 2017). PPI is related to neurological disorders like schizophrenia, obsessive compulsive disorder or Tourette syndrome, being characterised by a loss of gating in motor, cognitive and sensory manner (Braff *et al.*, 2001). For the testing of the ASR and the PPI, the Acoustic Startle Reflex Starter Package for Rat or Mouse and corresponding materials of Med associates were used. About 10 minutes before testing, mice were put into an animal holder (figure 4.3 A), which enclosed the animal. In order to avoid that animals will escape from animal holders, tape was used to fix the doors properly. For testing, mice were placed separately into the sound attenuating cubicle (figure 4.3 B). The cubicle was equipped with Styrofoam to diminish noise and consisted of a startle platform with a load cell on top. The animal holder with the locked mouse was fixated on that load cell. The load cell transduced the measured force and created an electrical signal in a direct proportional manner. From this, it followed that during the testing time, all movements of the mice were recorded. The platform with load cell was connected to the startle platform attenuator, which included a loud speaker to produce the background noise of 65 dB and a loud speaker for the different acoustic stimuli. Each animal was tested for about 1 hour and 16 minutes, whereat up to eight animals were tested simultaneously in different boxes.

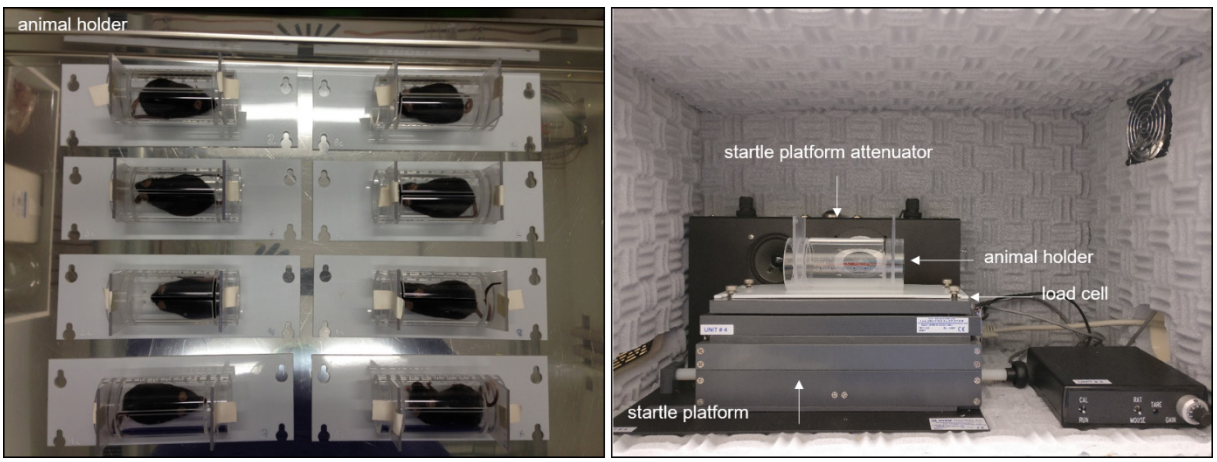

Figure 4.3: Experimental setup for acoustic startle reflex and prepulse inhibition testing. (A) Overview of eight animals sitting in an animal holder, which is closed on both sides by doors. The doors are fixated with tape. **(B)** Overview of the sound attenuating cubicle without a testing mouse. Inside of the cubicle, the animal holders are fixated on the load cell, which is fixated with the startle platform. Movements of the mice are transmitted as an electrical signal to the startle platform attenuator. The startle platform attenuator includes loud speakers for the background noise and the particular stimuli. Picture (B) was taken by the technician Jan Einicke from Institute of Developmental Genetics at the Helmholtz Zentrum München.

Two different approaches were tested: the ASR and the PPI. For the ASR testing, animals were exposed to stimuli with different sound pressure levels: null stimulus (NS), 70 dB, 80 dB, 85 dB, 90 dB, 100 dB, 110 dB and 120 dB. Henceforth these stimuli are called single stimulus (**SS**, figure 4.4). Testing the NS was necessary to consider the normal body movement of the mice while being exposed to a background white noise of 65 dB. Each SS was composed of white noise with a duration of 40 ms. In order to analyse the PPI, animals were exposed to a 110 dB white noise stimulus (**SP**, figure 4.4), which was proceeded by a prepulse tone with a frequency of 12 khz (**PP**, figure 4.4). The sound pressure of the PP differed and included the following values: 67 dB, 69 dB, 73 dB and 81 dB with a duration of 10 ms respectively. The interval between the PP and SP was 50 ms. Testing session started with a 5 min long acclimation period. Followed by five consecutive SS with 110 dB, which were not taken into account for analysis. Afterwards, SS and SP stimuli with different sound pressure level alternated in a random manner whereat each trial was exposed for ten times, organised in ten blocks with each trial type occurring once per block. The inter-trial interval varied between 20 s to 30 s.

In order to analyse the data, the maximal peak-to-peak amplitudes were taken to define the reflex. The corresponding software of the used hardware analysed automatically the maximum peak values and expressed it in arbitrary units. The PPI was calculated by taking the difference of the maximum peak values caused by the SS with 110 dB and SP (110 dB) with different prepulse sound pressures, set in relation to the maximum reflex peaks of SS (110 dB) and expressed in percentage (formula, figure 4.4).

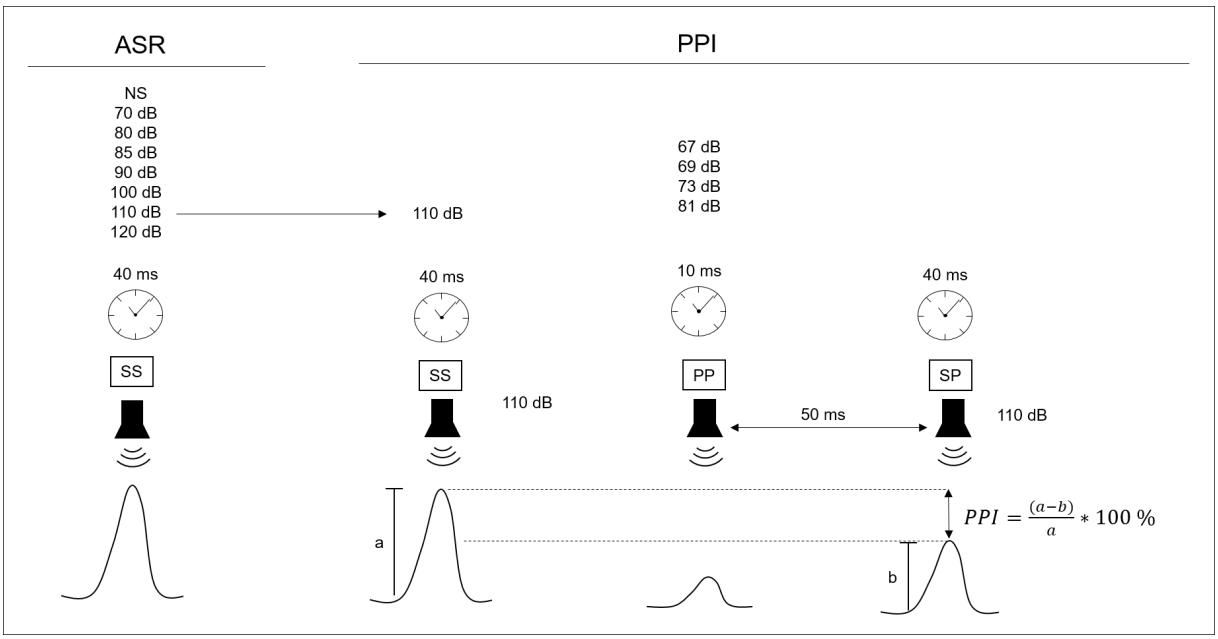

Figure 4.4: Schematic flowchart of acoustic startle reflex and prepulse inhibition. To test the ASR, animals are exposed to single stimuli (**SS**) with different sound pressure levels: 70 dB, 80 dB, 85 dB, 90 dB, 100 dB, 110 dB, 120 dB with a stimulus duration of 40 ms. Each acoustic stimulus causes a certain reaction of the animals, schematically represented in the curve. To test the PPI, animals are exposed to a 40 ms lasting stimulus with a sound pressure of 110 dB (**SP**) including a proceeded prepulse (**PP**). The prepulse differs in sound pressure: 67 dB, 69 dB, 73 dB and 81 dB and lasts 10 ms. The time interval between the PP and SP takes 50 ms. The PPI is calculated by subtracting the maximum peak value of the curve caused by the SS with 110 dB (**a**) with the maximum peak value of the curve caused by the SP (**b**), putting it into the ratio to the maximum peak value of SS and expressing it in percentages.

4.8.3 CatWalk testing (CW)

Since Parkinson's disease (PD) is typical a movement disorder, animals gait and movement were tested. Hereby, the automatic gait analysis system Catalk XT Noldus was used because it enables animals to walk freely without forcing animals to move, which resembles more the natural movement. The CatWalk system is of advantage since animals are able to walk at the speed that their gait (ab)normalities or endurance will allow (Batka *et al.*, 2014). In order to analyse the gait or movement, mice went through a testing with the CatWalk XT Noldus system. Animals were able to run at their own intrinsic speed in an unforced manner through a guided corridor whereat the contact areas and the body contour of the animals was videotaped by giving information about the gait of the animals (Hölter and Glasl, 2012).

The CatWalk XT system is composed of a glass plate, which was surrounded by black plastic walls to form a corridor with the dimensions of 130 x 68 x 152 cm (L x W x H). The endings of the corridor were open to enter and exit the walkway (figure 4.5). The walkway formed the part of the corridor, which was videotaped, with the dimensions of a length and width of about 35 x 9 cm. This size enabled to videotape about 4 - 5 step

cycles per run. The ceiling of the walkway was illuminated by red light (625 nm red LED) with a voltage of about 18 V. Red light was used to avoid distracting mice by bright light since mice have a dichromatic colour vision and are not able to detect the red colour (Jacobs *et al.*, 2007). However, the red lights enabled the experimenter to see the body contour of the mouse, which was necessary to detect the correct classification of the paws. Underneath the glass plate, a high-speed video camera (100 fps) was positioned to videotape and to detect the contact areas of the glass plate. This was possible by green light (535 nm green LED) which illuminated the glass plate with a voltage of 15 to 16 V. After contacting the glass plate, the green light was scattered and videotaped. The horizontal and vertical size of a single pixel was about 0.78 mm. The camera was connected to the computer. Each recorded pixel gave information about brightness, which allowed the experimenter to get information about intensity of the different paws. In addition, the CatWalk XT system software was also able to interpret other parameters related to the paws and the spatial and temporal relationship between the paws.

Each day before testing, light in the testing room was set to 80 Lux. To motivate animals to run over the walkway, the home cage was set at the end of the corridor since mice smelled their home and the cage members. The home cage was covered by a black goal box to keep mice away from light. In addition, to avoid other mice entering the corridor by climbing out of the home cage, the cage itself was covered by a stainless steel lid of the cage type II PC (figure 4.5). Each run started with the placement of one front paw on the walkway and finished with leaving the walkway with one of the hind paw. Animals were not trained before testing, however, the first runs were not considered in the data analysis since mice´ gait was different for the first trials. After passing the walkway, mice were taken out of the goal box and re-placed at the starting of the corridor. In the best case, mice exhibited a minimum of five “good runs” characterized by a consistent movement without interruptions or hitches. In case, one mouse needed more than circa 45 min to achieve the five “good runs”, the testing of that mouse was finished and re-done at another time point to avoid that the mouse felt asleep on the glass plate or felt exhausted.

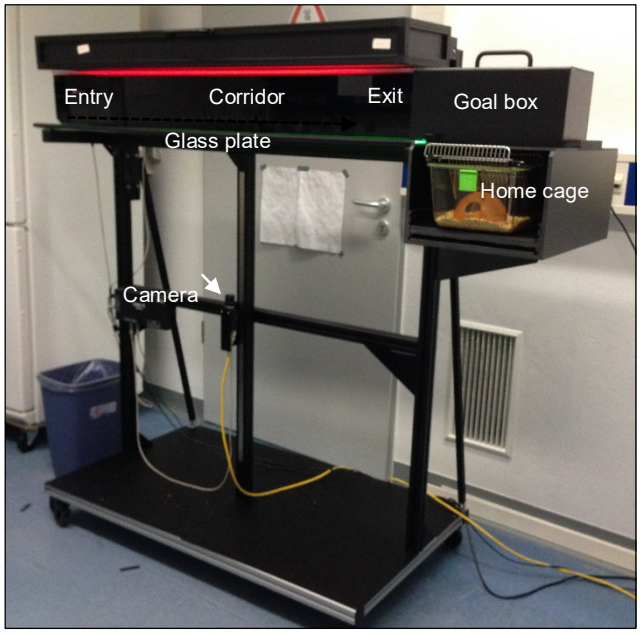

Figure 4.5: Experimental setup of CatWalk XT. Mice are put on the entry of the corridor to run over the glass plate, which is illuminated by green LED light to enable the camera (indicated by the arrow) to record the paw of the mice. The exit of the corridor ends in the goal box, which covers the home cage, to motivate the mice to run over the walkway. The corridor is illuminated by red LED light to visualise the recorded movie and to detect front prints.

A few days before the CatWalk testing procedure all mice were weighed to get information about the average span of weights between the different groups and sex. The average weight was used to set up the green light intensity in the programme CatWalk XT. This was done by putting an animal with an average weight on the glass plate followed by adjusting the camera gain and green intensity threshold. The younger animals were recorded with a camera gain of around 13 dB, compared to the older animals, which were videotaped with a camera gain of 8 to 9 dB. The green intensity threshold ranged between 0.05 and 1.4.

Afterwards, run criteria were set to define a run as compliant or not compliant. The minimum run duration was set to 0.5 seconds followed by the maximum run duration of 10 seconds. In addition, the maximum variation of speed was set to 40% including an average speed between 30 cm/s and 90 cm/s because intensive speed variation could cause problems in standardisation (Batka *et al.*, 2014). The minimum numbers of consecutive steps were set to a number of 10. The green and red light were set to automatic detection and were almost identical during the testing of different mouse cohorts (see above). Since mice contaminated the glass plate by excrement and urine, the plate needed to be cleaned several times between CatWalk testing. To avoid some background being measured as a paw, the background was snapped several times especially after cleaning. For the analysis, four out of the five compliant runs were

analysed in order to avoid high variation within one mouse. In some few cases, mice did not perform well on running over the walkway and a total number of five or four “good” runs could not be videotaped. In that case, a minimum of three runs were considered for analysis per mouse. Next to the run criteria, each movie was double checked for the quality of the paw prints. Since the camera frequency rate was 100 frames per second and animals needed about 2 s to pass the walkway, a movie was composed of around 200 pictures. The position of the paw prints could be detected and labelled automatically via the use of the automatic footprint classification (AFC). However, often some paws needed to be re-labelled manually due to some mismatches of two paws. In case the background disturbed the AFC, the green intensity threshold was increased manually for each run

4.8.4 Olfactory testing (Olfac.)

In order to test the olfactory ability, mice went through a testing of discrimination of binary mixtures and sensitivity testing of a diluted odorant (Glasl *et al.*, 2012, Schellinck *et al.*, 2001). Female mice were not tested because previous published literature did not show indications of an impairment in olfaction of female *Pink1* KO mice (Glasl *et al.*, 2012). This suggests that female mice lacking Pink1 in serotonergic or dopaminergic neurons might not exhibit olfactory impairments.

During the training, conditioning and olfactory testing, mice were kept singly in a type IIL mouse cages filled with some shavings material. A stainless steel lid and a soft lid covered the cages. Before the testing, animals were habituated for 15 min in the testing cage. The mice were placed on a restricted feeding regime to maintain their body weight at around 90% body weight of their free-feeding level. The body weight restriction started three days before the training and kept on going for the whole of the training and conditioning procedure including all olfactory testing.

During the experiment, one or two dishes were horizontally placed on a T-carrier, which were separated by a vertical barrier (Figure 4.6 A). For each trial, dishes were filled with fresh scented shavings. When two dishes were used, both were presented randomly to avoid a side effect. For each trial, the T-carrier was put to the front part of the testing cage. The mice were separated from the T-carrier by a cage barrier inside the testing cage (figure 4.6 B). Afterwards, the cage barrier was taken out of the cage (figure 4.6 C). The behaviour of the mouse was observed followed by a decision by the experimenter, e.g. the mouse dug into the correct dish and was rewarded by a

chocolate piece (figure 4.6 D & E). Then, the T-carrier was pulled out of the cage (figure 4.6 F) and the cage barrier was put back into the testing cage ensuring that the mouse is in the back part of the cage.

Before the olfactory testing, mice needed to go through a three to four day long **1) training phase** to get used of the taste of chocolate and to dig in a dish filled with shavings of chocolate (figure 4.7 (1)). To enable this, three chocolate pieces were put on top of the shavings in one dish. After the mice ate the chocolate, new chocolate pieces were presented. The chocolate pieces were presented for five times. On the next day, three chocolate pieces were placed on top, in the middle and at the bottom of the dish filled with bedding. The mice needed to bury to reach the hidden chocolate pieces. After three times of digging for the chocolate, only two chocolate pieces were hidden in the shavings. The mice could not see the chocolate piece anymore. However, the mice needed to dig for an additional six times. On the next day, only one piece of chocolate was buried in the shaving and the mice needed to grub for six times. If the mice did not dig well enough, an extra training day was added.

Subsequently, the **2) conditioning phase** followed, where mice learned to connect the food reward with the odorant [S+] (figure 4.7 (2)). For the first two days, mice needed to differentiate between the odorant 2-Phenethyl acetate, defined as [S+] and the solvent Diethylphthalat. The odorant [S+] was diluted with the solvent to a 10% (v/v) working concentration. Two dishes were presented, one dish filled with scented shavings of [S+] and the other dish filled with solvent soaked shavings. Both dishes were immersed in a ratio of 1 ml of odorant or solvent per 3 g shavings. This ratio was consistent for the following testing. For the first three trials, a chocolate piece was placed on top of the dish with the [S+] odorant whereas the dish with solvent was not covered by a chocolate piece. The mice needed to eat the chocolate and connect the chocolate with the odorant [S+]. For the next six trials, a chocolate piece was hidden in the dish with the odorant [S+] and in the dish with the solvent. On the next day, the last six trials were repeated. For the completely conditioning phase, mice were only allowed to dig in the dish with the [S+] odorant and to eat the chocolate. In case the mice dug in the dish with the solvent, the trial was stopped. In addition, it was prohibited to let the mice eat the chocolate in case of choosing the dish with the solvent.

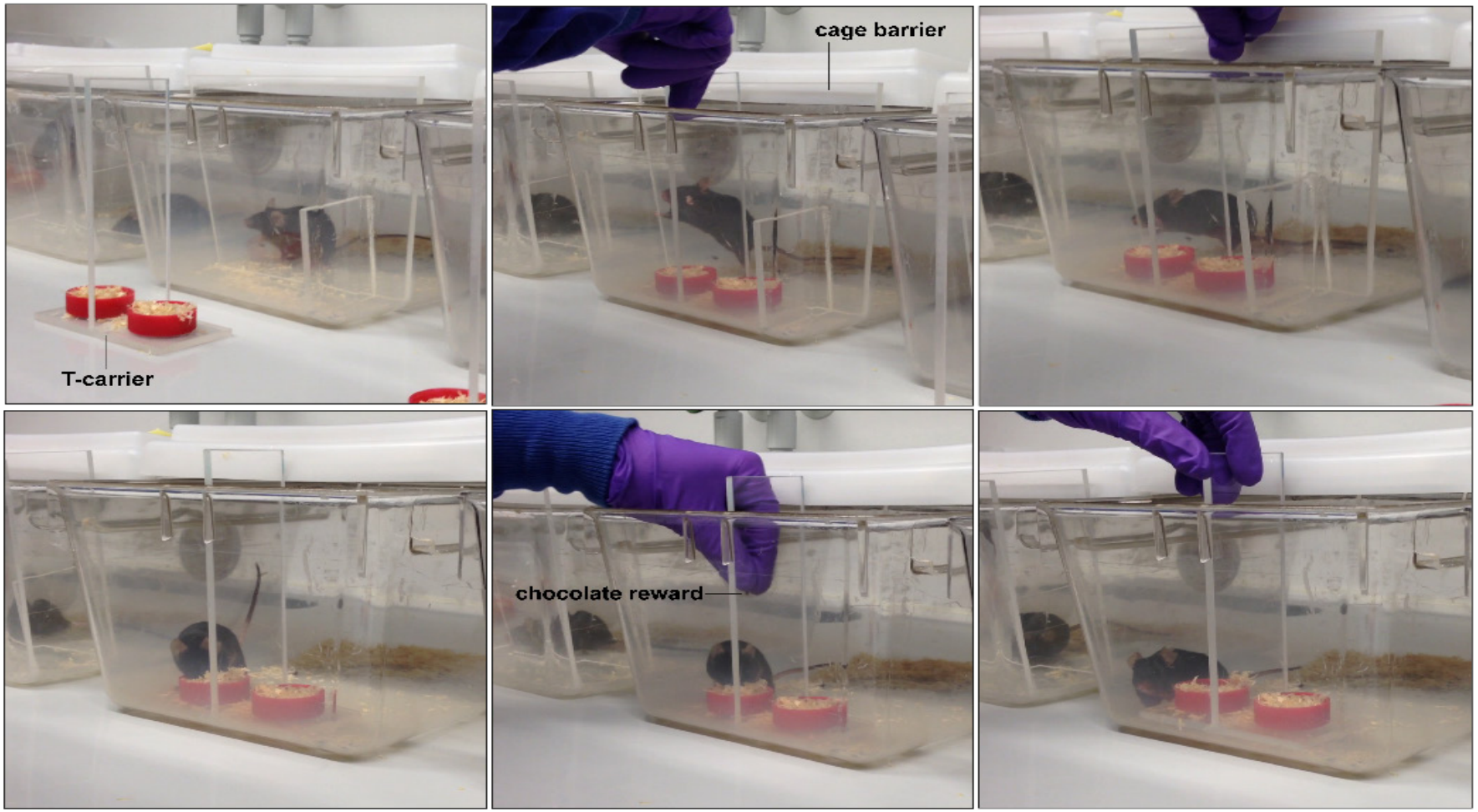

Figure 4.6: Sequence of the binary mixture testing. (A) Two dishes are horizontally placed on a T-carrier. **(B)** The T-carrier is put in the front part of the testing cage. The mice are separated from the T-carrier by a cage barrier inside the testing cage. **(C)** Afterwards, the cage barrier is taken out of the cage. **(D)** The mouse dugs into the dish. **(E)** In case of correct choice, the mouse is rewarded by a chocolate piece. **(F)** Then, the T-carrier is taken out of the cage.

Next, the mice needed to discriminate between a dish scented with the odorant [S+] and a dish scented with the odorant [S-], Methyl trans-cinnamate (figure 4.7 (2)). Both odorants were diluted in a 10% (v/v) working concentration. The mice were previously trained to dig in a dish with the odorant [S+]. Firstly, a piece of chocolate was buried on top of the dish with [S+] where the mice needed to dig for the chocolate for six trials. Afterwards, the chocolate was placed on both dishes with the odorants [S+] and [S-]. Digging in the dish with the odorant [S+] was considered as the correct choice. Digging in the dish with the odorant [S-] was contemplated as the wrong choice, the trial was stopped, and the mice were prohibited to eat the chocolate piece.

The actual testing started with the **3) binary mixture discrimination test** where mice needed to differentiate between a mixture of the odorants [S+] and [S-] (figure 4.7 (3)). Both odorants were diluted to a working concentration of 10% (v/v). This working concentration was defined as 100% of the particular odorant. The dish containing the odorant [S+] is called “dish I”. The dish containing the odorant [S-] is defined as “dish II”. The odorants [S+] and [S-] were mixed in consistent steps to make the odorants increasingly similar (table 4.2).

Table 4.2: Mixture of odorants in binary discrimination test.

dish I	dish II
% [S+] : % [S-]	% [S-] : % [S+]
100 : 0	0 : 100
70 : 30	70 : 30
55 : 45	55 : 45
53 : 47	53 : 47
51 : 49	51 : 49

The correct choice of the mouse was defined by its first indication to dig in the dish I with a higher concentration of [S+] and rewarded by a given piece of chocolate. In case the mouse failed in the discrimination of [S+], both dishes were immediately removed, the trial was finished and evaluated as incorrect choice.

Subsequently to the binary mixtures discrimination test, the **4) sensitivity test** was performed (figure 4.7 (4)). Here, mice needed to choose between a “dish III” and “dish IV”. The “dish III” was scented with the stepwise diluted odorant [S+] compared to “dish IV” scented with solvent. The 10% (v/v) working concentration of [S+] was stepwise binary diluted with the solvent thus a 5% (v/v) concentration of [S+] is the first dilution step followed by the second dilution step of 2.5% (v/v) concentrated [S+] and further on (table 4.3). The correct choice of the mouse was defined by its first indication to dig in the “dish III” scented with the odorant [S+] and rewarded with a given piece of chocolate. In cases where the mouse failed to detect the diluted odorant [S+], both dishes were removed immediately. The trial was finished and evaluated as incorrect choice. After three correct responses in a row, the mouse reached a higher dilution step followed by a lower concentration of [S+]. If a mouse dug twice in a row in the wrong “dish IV”, the mouse was re-tested with the one step higher concentrated dilution step until the mouse reached the three correct choices again. The highest dilution step with three successful in correct choices of “dish III” was defined as the sensitivity threshold.

Table 4.3: Different dilution steps of [S+] in sensitivity test.

dilution steps	% [S+] (v/v)	concentration [S+] (v/v)
stock	100.00	100
working concentration	10.00	1.0E+01
1	5.00	5.0E+00
2	2.50	2.5E+00
3	1.25	1.3E+00
4	0.63	6.3E-01
5	0.31	3.1E-01
…	…	…

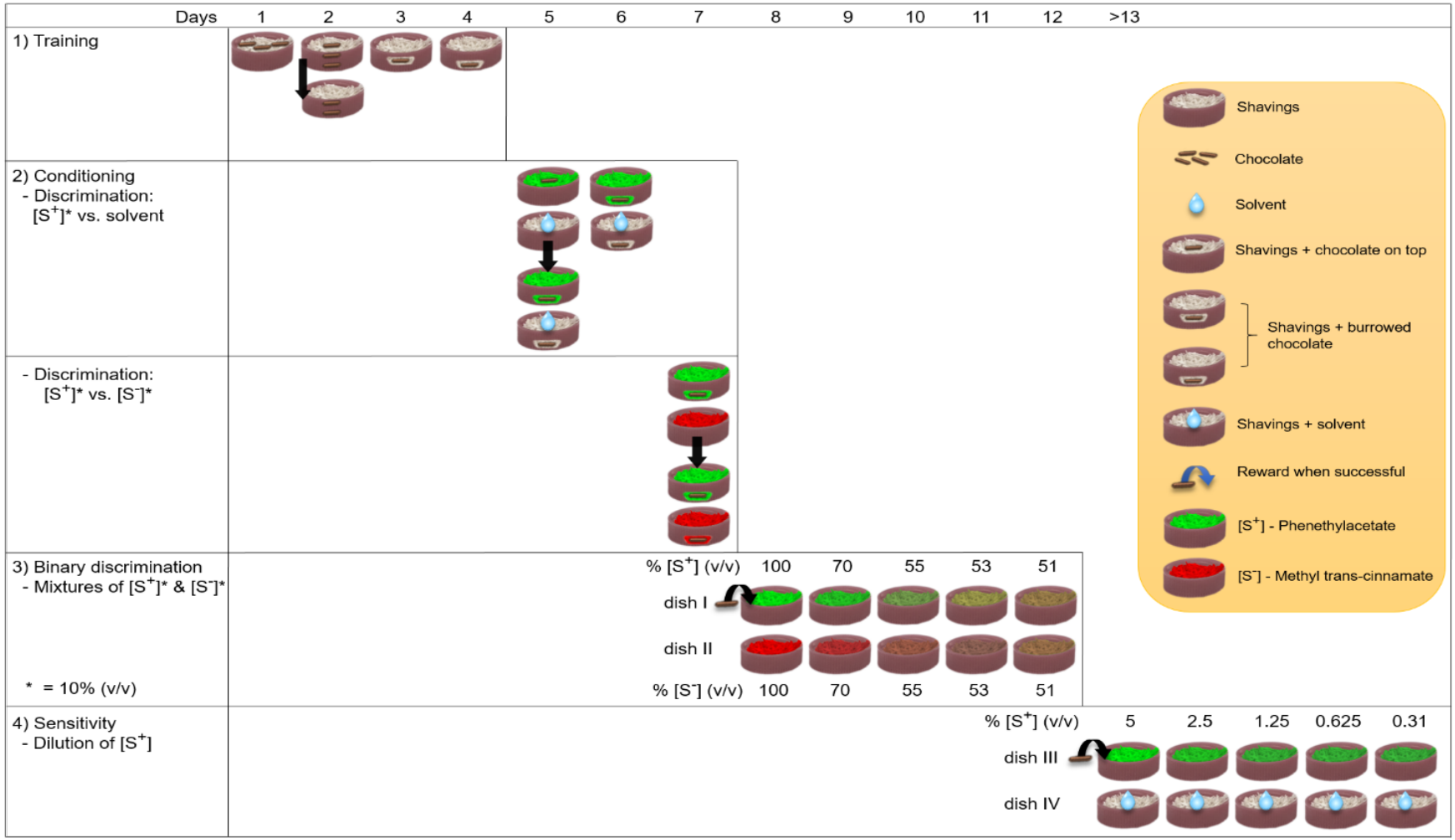

Figure 4.7: Process of the training, conditioning, discrimination test of binary mixtures and sensitivity test. The tested mice need to run through a training and conditioning before the actual olfactory testing are performed. At the first training days, the mice need to get used to the taste of chocolate and to learn to dig for the chocolate. Afterwards, the mice are conditioned to connect the chocolate with the odorant [S+] and to discriminate between the odorants [S+] and the odorant [S-]. Thereupon in the discrimination test of binary mixtures, mice need to differentiate two stepwise-diluted mixtures of the two odorants [S+] and [S-]. At the end, the mice are tested on the sensitivity testing where the mice need to discriminate between a dish with solvent compared to the stepwise diluted odorant [S+].

4.9 Statistics

For all statistical testing, the programs SPSS and GraphPad Prism were used. The independent variables were defined by sex and genotype. All statistical tests were performed in a two-tailed manner. A statistical significance was defined when $p < 0.05$ =*; $p < 0.01$ = ** and $p < 0.001$ = ***. The aim was to determine possible genotype effects with significant differences between the mutant mice compared to both tested controls. Only in case the values of the dependent variables of both controls differed significantly from those of the mutant mice, these variables were interpreted as a genotype effect caused by the KO of *Pink1* in the serotonergic or dopaminergic neurons. However, it was important to consider that the two independent variables could possibly interact. Therefore, in addition, the sex was always analysed separately. The multiple comparisons of different genotype groups were done by the post hoc Bonferroni test.

For the quantification of dopaminergic neurons and the selected neurotransmitter, three groups of genotypes per sex were analysed (control 1: Cre:Cre+; Pink1:wt/wt; mutant: Cre:Cre+; Pink1:flox/flox and control 2: Cre:Cre-; Pink1:flox/flox). In sum, the six groups were analysed via the 2-way analysis of variances (ANOVA) test, where the number of dopaminergic neurons and selective neurotransmitter formed the single dependent variable.

For the quantification of serotonergic fibers, two groups of genotypes per sex were tested (mutant: Cre:Cre+; Pink1:flox/flox and control 2: Cre:Cre-; Pink1:flox/flox). In sum, the two groups were analysed via the 2-way ANOVA, where the density of serotonergic fibers formed the dependent variable.

In the OF, ASR/PPI and CatWalk testing, the three genotype groups per sex were analysed (control 1: Cre:Cre+; Pink1:wt/wt; mutant: Cre:Cre+; Pink1:flox/flox and control 2: Cre:Cre-; Pink1:flox/flox). In sum, the three groups were analysed via the 2-way ANOVA test, where one of the several parameters of each test formed the single dependent variable.

In the olfaction testing, the three genotype groups of male mice were analysed (control 1: Cre:Cre+; Pink1:wt/wt; mutant: Cre:Cre+; Pink1:flox/flox and control 2: Cre:Cre-; Pink1:flox/flox). In sum, the three groups were analysed via the 1-way ANOVA test, where the dependent variable formed on the one hand the percentage of correct choices in binary mixture testing and on the other hand the sensitivity threshold.

Chapter 5

Results

Since *Pink1* full knockout (KO) (Pink1_del2/3) mice showed evidence of slight motor and definitive non-motor symptoms of Parkinson's disease (PD) (Glasl *et al.*, 2012), the major aim was to dissect the influences of distinct neurotransmitter systems on these PD-related phenotypes. For this purpose, transgenic mice with a specific knockout of *Pink1* in serotonergic or in dopaminergic neurons were generated: Pink1_CKO x Pet1_Cre_TG (Pink1 x Pet) and Pink1_CKO x Slc6a3_CreERT2_TG (Pink1 x DAT) (material 3.9). Using this approach, also possible systemic compensatory mechanisms at play in the Pink1_del2/3 mice (Glasl *et al.*, 2012) might be overcome. After validation of the specific KO of *Pink1* in serotonergic or dopaminergic neurons, these mouse lines underwent a set of behavioural testing: open field (OF), acoustic startle reflex (ASR) and prepulse inhibition (PPI), CatWalk (CW) and olfaction test (Olfac.) (figure 5.1, blue squares). A dominant pathological hallmark of PD is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNC) (Fearnley and Lees, 1991), which was not detectable in the Pink1_del2/3 mice (Glasl *et al.*, 2012). To determine whether the specific KO of *Pink1* in the particular neurotransmitter system thus leads to this parkinsonian phenotype, immunohistochemical staining for tyrosine hydroxylase (TH) was performed and dopaminergic neurons in the SNC were quantified stereologically. In addition, the neurotransmitter content of certain brain regions was analysed via high performance liquid chromatography (HPLC) measurements. As Pink1_del2/3 mice exhibited an impaired serotonergic innervation into the olfactory bulb (OB) (Glasl *et al.*, 2012), the OB of the Pink1 x Pet and Pink1 x DAT mice were stained immunohistochemically for serotonin (5-HT) and serotonergic fibres were quantified stereologically (figure 5.1, green squares). In addition, because PD is an age-related disease (Poewe *et al.*, 2017), mice were analysed behaviourally at an age of 4 - 7 months and re-tested at an age of 14 - 18 months, henceforth defined as young and mid-aged animals (cohort 1). In order to obtain information about the neuroanatomy and physiology of young

animals, a separate cohort was analysed, which did not undergo behavioural testing (cohort 2) (figure 5.1).

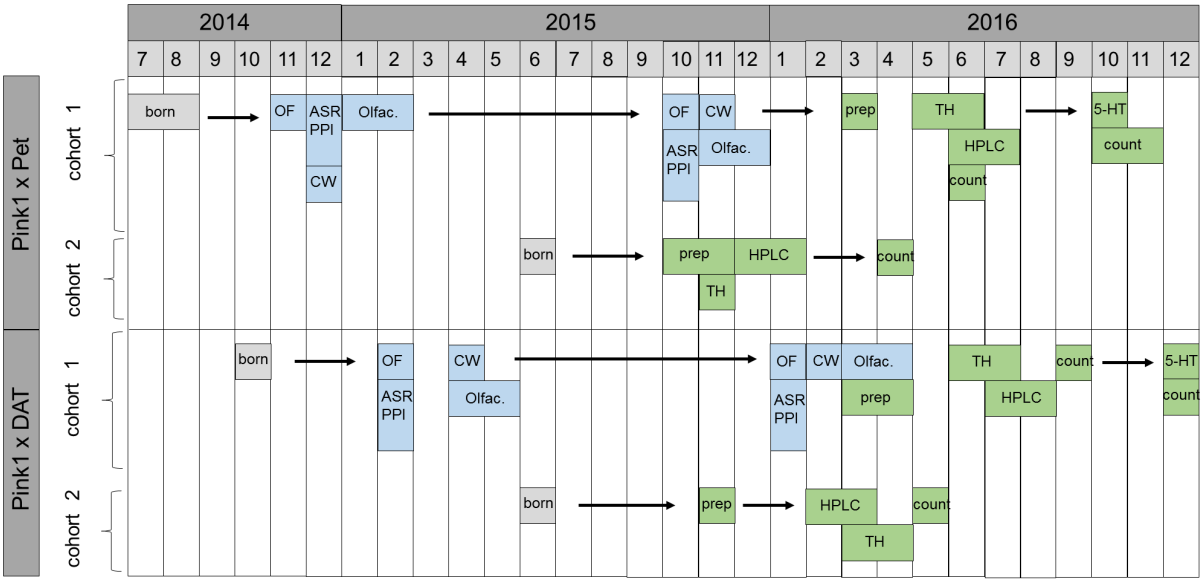

Figure 5.1: Schedule of analysis of the Pink1 x Pet and Pink1 x DAT mice based on behavioural, neuroanatomical screening. Pink1 x Pet and Pink x DAT mice of the cohort 1 were born in summer/autumn 2014 and behaviourally tested with a young age of 4 - 7 months (blue squares). Afterwards mice were re-tested with mid-age of 14 - 18 months (blue squares), followed by immunohistochemical staining of TH^+ and $5\text{-}HT^+$ cells and neurotransmitter quantification via HPLC measurements (green squares). In order to obtain neuroanatomical data from young mice, additional cohorts were used, labelled as cohort 2. These mice were born in autumn 2014 and summer 2015 and analysed with an age of 4 months. OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition, CW = CatWalk, Olfac. = olfaction test, prep = preparation of mouse organs, TH = staining of tyrosine hydroxylase positive cells, HPLC = identification of the neurotransmitter content via high performance liquid chromatography, 5-HT = staining of serotonin positive cells, count = quantification of immuno positive cells, black arrows indicate the scheduling.

In total, 73 mice of the mouse line Pink1 x Pet and 69 mice of the mouse line Pink x DAT were initially tested for behavioural changes at young ages. Over time, the number of mice was reduced due to genotype-independent illness or age-related dying. Thus, 67 mid-aged mice of the mouse line Pink1 x Pet and 64 mid-aged mice of the mouse line Pink1 x DAT underwent the behavioural testing. A total number of 41 - 46 mice were considered for the analysis of TH^+ cells in the SNC in young and mid-aged mice of both mouse lines. 48 animals were analysed in order to detect the neurotransmitter content of young and mid-aged Pink1 x Pet and Pink1 x DAT mice. The serotonergic innervation into the OB was only researched in mid-aged mutant (mt) and control 2 (ctrl.2) mice, with 8 - 12 mice per mouse line (figure 5.2). A detailed overview of all tested mice is shown in the appendix in tables A1 - A10.

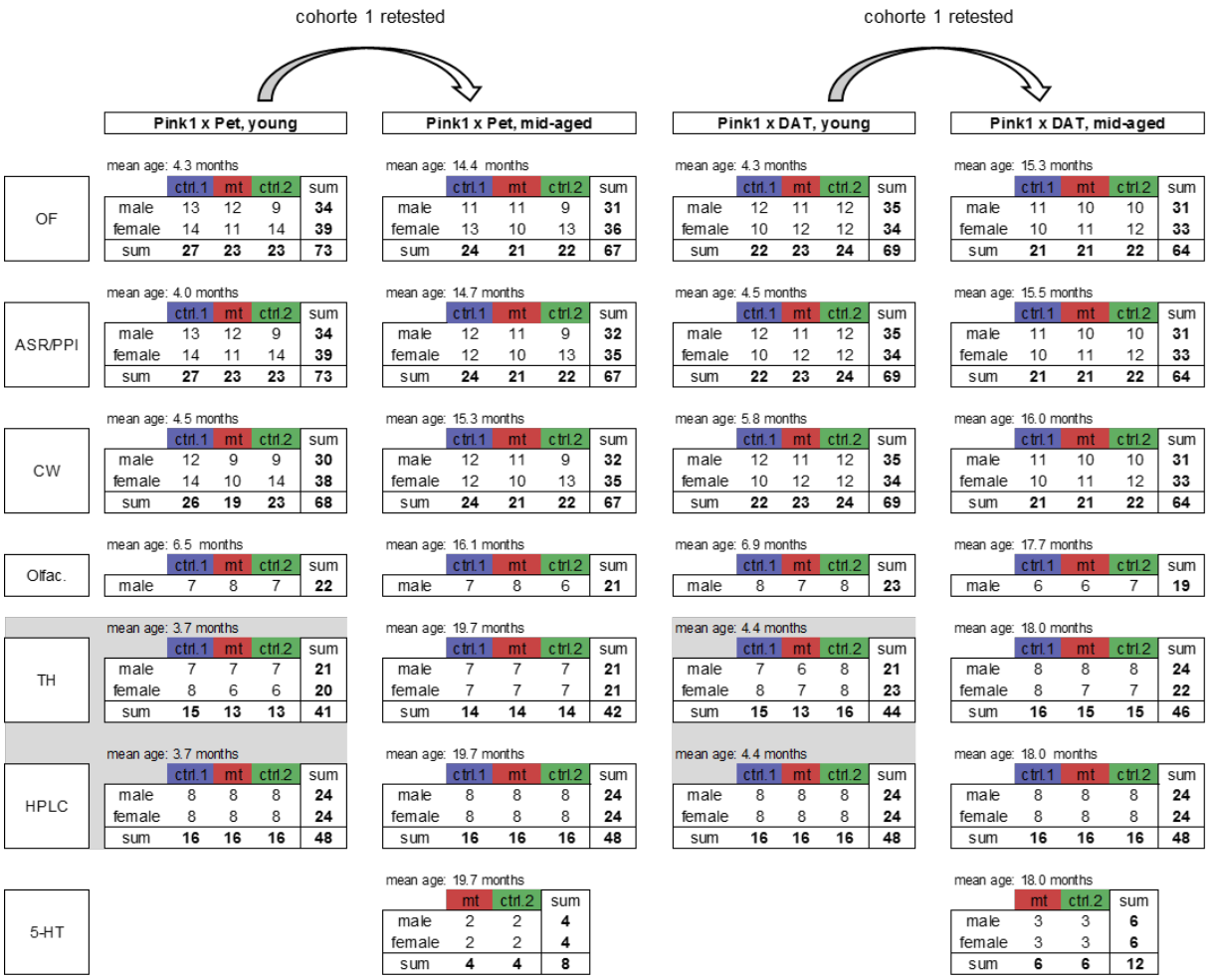

Figure 5.2: Total number of analysed animals. Pink1 x Pet and Pink1 x DAT mice were analysed with young and mid-age in behaviour, neuroanatomy and neurotransmitter content. Two cohorts per mouse line were used (cohort 1: white background, cohort 2: grey background). OF = open field ASR = acoustic starle reflex, PPI = prepulse inhibition, CW = CatWalk, Olfac. = olfaction, TH = quantification of TH^+ cells, HPLC = quantification of neurotransmitter content via HPLC, 5-HT quantification of $5\text{-}HT^+$ cells.

The interest was to figure out possible genotype effects with Pink1 deficiency in serotonergic and dopaminergic neurons. The Pink1 x Pet mouse line was generated by crossing a Cre recombinase expressing mouse line (under a Pet1 promotor) with conditional *Pink1* mice (Scott *et al.*, 2005, Glasl *et al.*, 2012) (materials 3.9.3). In addition, the Pink1 x DAT mouse line originated by crossing a mouse line expressing a tamoxifen-induced form of Cre recombinase (which was cloned in the *Slc6a3* gene) with the conditional *Pink1* KO line (Rieker *et al.*, 2011, Feil *et al.*, 2009, Glasl *et al.*, 2012) (materials 3.9.4). The resulting Cre recombinase expressing mice, which were homozygous for the floxed *Pink1* were defined as mutant mice (mt), labelled as Cre:Cre+; Pink1:flox/flox. In order to control for a potential Cre recombinase effect and an effect due to the construct in *Pink1*, two control groups were considered for the analysis:

- control 1 (ctrl.1): Cre:Cre+; Pink1:wt/wt
(expression of Cre recombinase and wild type *Pink1*)

- control 2 (ctrl.2): Cre:Cre-; Pink1:flox/flox
(absence of Cre recombinase and presence of floxed exon 2 and 3).

Parameters in the mutant mice were only considered statistically significant when they differed significantly compared to both control groups. It is important to note that significant differences in parameters between the two control groups or, more importantly, between only one control group and the mutant mice were regarded as being irrelevant for Pink1 function in the respective neurotransmitter system and were thus neglected. These differences might have occurred due to either a Cre-effect per se, or more likely the presence of the floxed allele. In order to figure out potential sex-specific genotype effects, each group was tested separately for males and females. Data from the olfaction test were statistically analysed with a 1-way ANOVA, followed by Bonferroni post hoc test. All other analyses were analysed statistically using a 2-way ANOVA test, followed by the Bonferroni post hoc test. When genotype effects were sex dependent, male and female data was shown separately. When there was no interaction between sex and genotype, data of males and females were pooled.

5.1 Validation of neuronal population specific recombination of *Pink1*

In order to prove the functionality of the conditional approach of the floxed *Pink1* and the Cre recombinase mediated deletion of exon 2 and 3, a PCR based genotyping in different tissues and brain regions was performed. For this purpose, samples were considered that included and excluded the neuronal populations targeted by the Cre-driven recombination, i.e. the serotonergic and the dopaminergic neurons. The PCR strategy was based on the detection of a 583 bp DNA fragment, by using the primer neocass_for_AH and ko_rev_AH in order to prove the deletion of exon 2 and 3 in a Pink1 deficient tissue (methods 4.2.1 and 4.2.2) (figure 5.3).

Figure 5.3: **Schematic overview of Pink1 in wild type, floxed and exon 2/3 deletion condition**. ***Pink1*:** wt, wild type *Pink1* is composed of 8 exons (purple). *Pink1*: flox, conditional knockout (CKO) of *Pink1* with flanked exons 2 and 3 by loxP sited. A neomycin cassette (neo, grey) is positioned upstream of the exon 2, flanked by FRT (blue) sites. *Pink1*: del2/3, after cutting of the active Cre recombinase on the loxP sites, the exons 2 and 3 and neomycin cassette are cut out, resulting in a *Pink1* knockout (KO). Primers are labelled, which were used for genotyping (table 3.5 and table 4.1).

The PCR product indicative of the floxed Pink1 alleles (688bp) could be detected in all tissues of the Pink1 x Pet and Pink1 x DAT mice, irrespective of the presence and absence of the Cre recombinase (figure 5.4, Pink1 flox PCR).

The anterior brainstem with the raphe nuclei is a region containing the most serotonergic neurons. Confirming the Cre-mediated recombination of *Pink1* in Pink1 x Pet mice specifically in this region, the PCR product representing the *Pink1* allele with a deletion in exon 2 and 3 (583 bp) could be detected only in this part of the brain. The selectivity of this recombination in serotonergic neurons was supported by the absence of the "deleted" allele in other brain regions, such as the cerebellum and cortex (cort.), where serotonergic neurons are sparse (figure 5.4, C).

The OB, midbrain (MB) and kidney (kidn.) contain dopaminergic neurons. Confirming the tamoxifen-induced Cre-mediated recombination of *Pink1* in these tissues in Pink1 x DAT mice, the PCR product representing the *Pink1* allele with a deletion in exon 2 and 3 (583 bp) could be detected in the OB, MB and kidney. The selectivity of this recombination in dopaminergic neurons was supported by the absence of the detection of this "deleted" allele in other brain regions, such as the hippocampus (HC) and cortex, where dopaminergic neurons are sparse (figure 5.4, C). In cases where the mice were not injected with tamoxifen, a Cre-mediated recombination was not detectable, proving the absence of leaky Cre recombinase (figure 5.4, K). In general, tamoxifen was injected at an age of 10 weeks and mice were firstly analysed at an age of about 4 months.

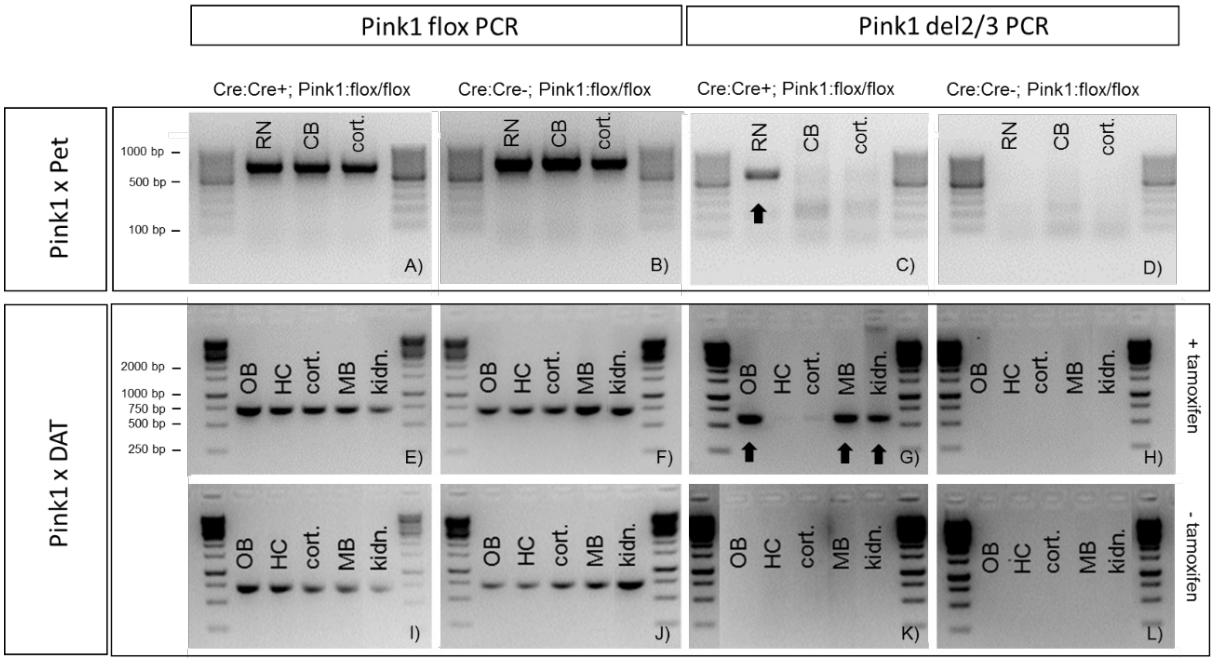

Figure 5.4: Proof of Pink1 deficiency in serotonergic and dopaminergic neurons in Pink1 x Pet and Pink1 x DAT mice. Pink1 flox PCR) The PCR product of the floxed *Pink1* allele could be proved in Pink1 x Pet and Pink1 x DAT mice. Pink1 del2/3 PCR) The PCR product for the Pink1 allele obtaining deleted exons 2/3 could be proved in the raphe nuclei (RN), the region with the most serotonergic neurons, of the Pink1 x Pet mice. The PCR product for the Pink1 allele obtaining deleted exons 2 and 3 could be proved in the olfactory bulb (OB, midbrain (MB) and kidney (kidn.), regions with dopaminergic neurons, of the Pink1 x DAT mice, however only in case of tamoxifen injection. In the absence of tamoxifen, the exons 2 and 3 were not deleted since the Cre recombinase did not show leakiness.

To summarize the PCR based characterization: The Pink1 x Pet and Pink1 x DAT mouse line exhibited a selective Cre recombinase driven recombination of the exons 2 and 3 in the *Pink1* allele in serotonergic and dopaminergic brain regions, respectively, thus highly likely in the respective neuronal populations. In addition, no recombination in the Pink1 x DAT mice was detectable if mice were not induced with tamoxifen. Thus, the inducible Cre-recombinase does not reveal gross ectopic activity in non-treated animals.

5.2 Neuroanatomical analysis

Since PD symptoms are still mainly regarded as being induced by a dysfunctional dopaminergic system, both mouse lines were analysed regarding the morphological integrity of this system. Furthermore, since in the Pink1_del2/3 mice a specific morphological impairment of the serotonergic neurons (i.e. the serotonergic innervation of the olfactory bulb was detected (Glasl *et al.*, 2012)) the integrity of this system was analysed as well. The following analysis was performed:

1. Quantification of the numbers of dopaminergic neurons in the SNC of the ventral midbrain (VM), to evaluate dopaminergic neuronal degeneration.

2. Determination of the tissue content of the both neurotransmitters and distinct metabolites in the target regions of these neurons, to evaluate possible dysfunction in dopamine (DA) and serotonin (5-HT) metabolism and release,

3. As well as the serotonergic innervation of the olfactory bulb, to evaluate dysfunctional serotonergic input into the OB.

5.2.1 Morphological integrity of the dopaminergic system

In order to analyse the number of dopaminergic neurons in the SNC, cryosections of both mouse lines were stained immunohistochemically using an anti-TH antibody as marker for this neuronal population. The number of positive neurons was determined by stereological means, at young and middle ages (methods 4.5 and 4.6).

No significant differences in the number of TH^+ cells in the SNC of the young and mid-aged mutant Pink1 x Pet and Pink1 x DAT mice were detected compared to the control groups. The statistics of the number of TH^+ cells is summarized in the ANOVA table A11 and shown in the appendix. A summary of the descriptive statistics is listed in table A12 in the appendix.

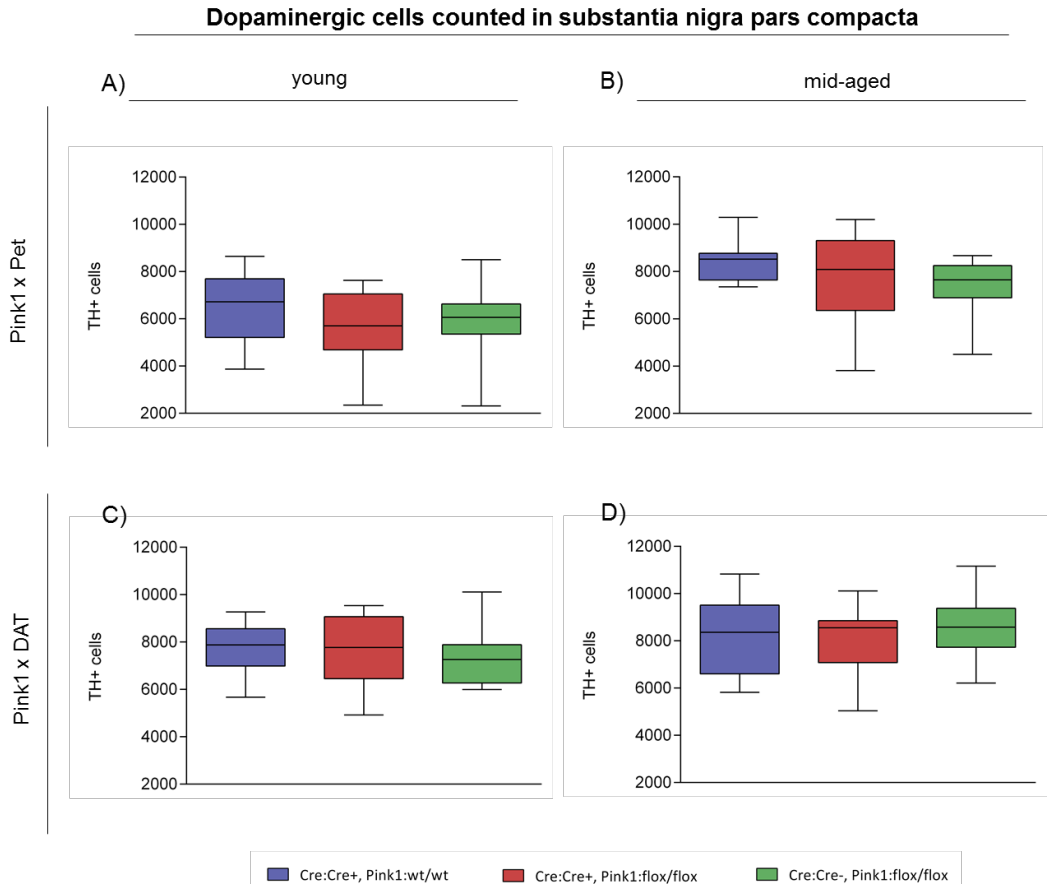

Figure 5.5: Number of dopaminergic neurons in substantia nigra pars compacta of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different genotype groups were tested indicated with different colours. All groups were tested in males and females and shown in a grouped manner. Blue = control 1: Cre:Cre+; Pink1:wt/wt. Red = mutant: Cre:Cre+; Pink1:flox/flox. Green = control 2:

Cre:Cre-; Pink1:flox/flox. (A & B) Pink1 x Pet mice. (C & D) Pink1 x DAT mice. Both mouse lines with a young age (A & C) and mid-age (B & D), did not exhibit significant differences in the number of TH^+ cells in the mutant mice compared to control mice. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

5.2.2 Tissue content of dopamine and serotonin in the target regions of the neurons

In order to analyse the content of DA, 5-HT and its metabolites in the OB, striatum (STR) and VM, brain tissue was purified and analysed via HPLC. The different substances: DA, 3.4-Dihydroxyphenylacetic acid (DOPAC), epinephrine (E), homovanillic acid (HVA), norepinephrine (NE), 3-Methoxytyramine (3-MT), 5-Hydroxyindole-3-acetic acid (5-HIAA) and 5-HT were considered for the analysis (methods 4.7). Epinephrine was not detectable in several mice, hence excluded from the analysis.

The analysis revealed no significant differences in the content of dopamine, serotonin and their metabolites in the three brain regions OB, STR and VM neither in young or mid-aged mutant Pink1 x Pet and Pink1 x DAT mice compared to the control groups (Figure 5.6 & 5.7). The statistical comparison for each substance is summarized in the ANOVA tables A13 – A16 and shown in the appendix. A summary of the descriptive analysis of the dopamine and serotonin content is listed in the table A17 & A18 in the appendix.

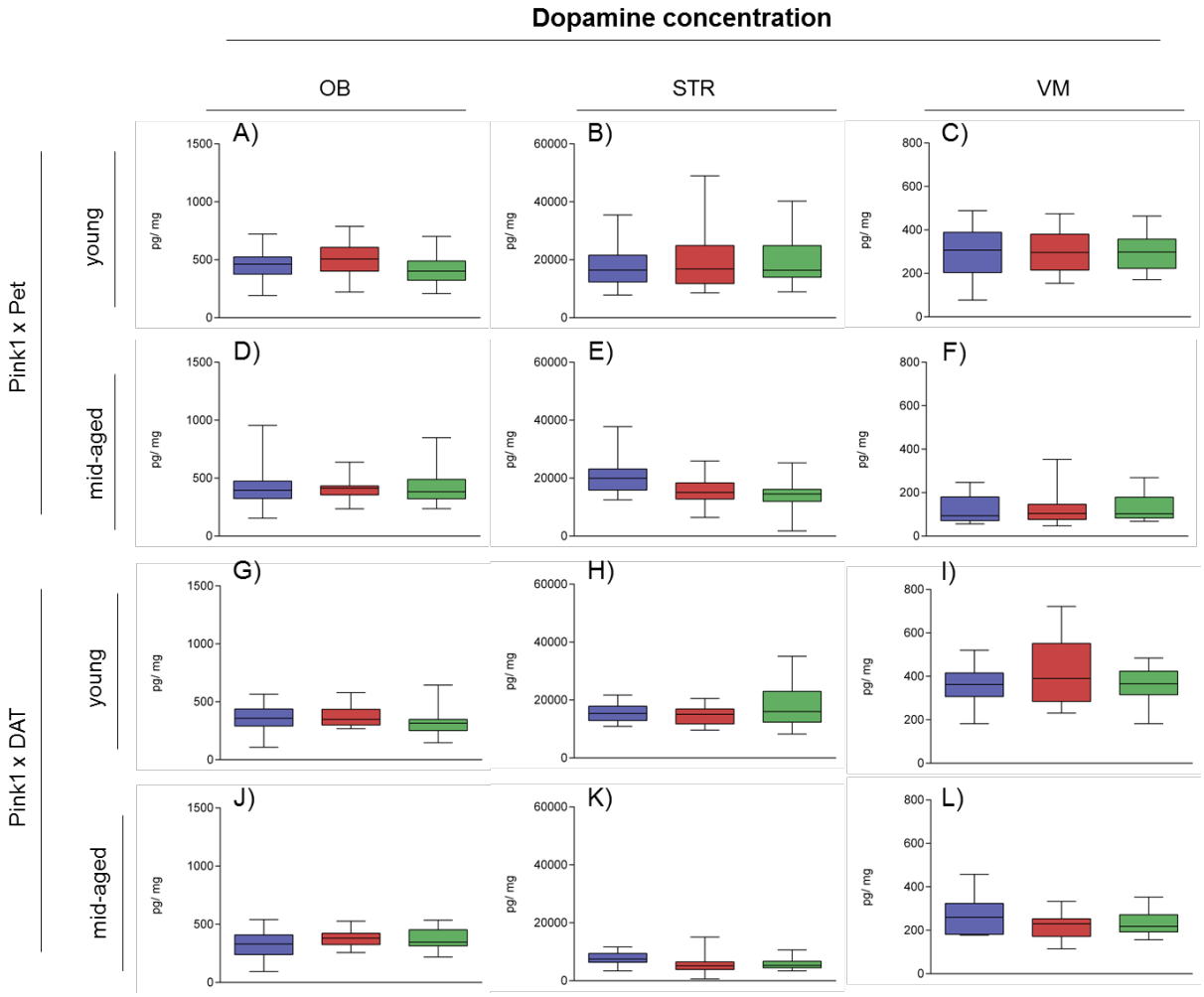

Figure 5.6: Dopamine content in olfactory bulb, striatum and ventral midbrain of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different genotype groups were tested indicated with different colours. All groups were tested in males and females and shown in a grouped manner. Blue = control 1: Cre:Cre+; Pink1:wt/wt. Red = mutant: Cre:Cre+; Pink1:flox/flox. Green = control 2: Cre:Cre-; Pink1:flox/flox. A - F) Pink1 x Pet mice. G - L) Pink1 x DAT mice. Both mouse lines with a young age (A - C, G - I) and mid-age (D - F, J - L) did not exhibit significant differences in the dopamine content in the olfactory bulb (OB), striatum (STR) and ventral midbrain (VM) in the mutant mice compared to control mice. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

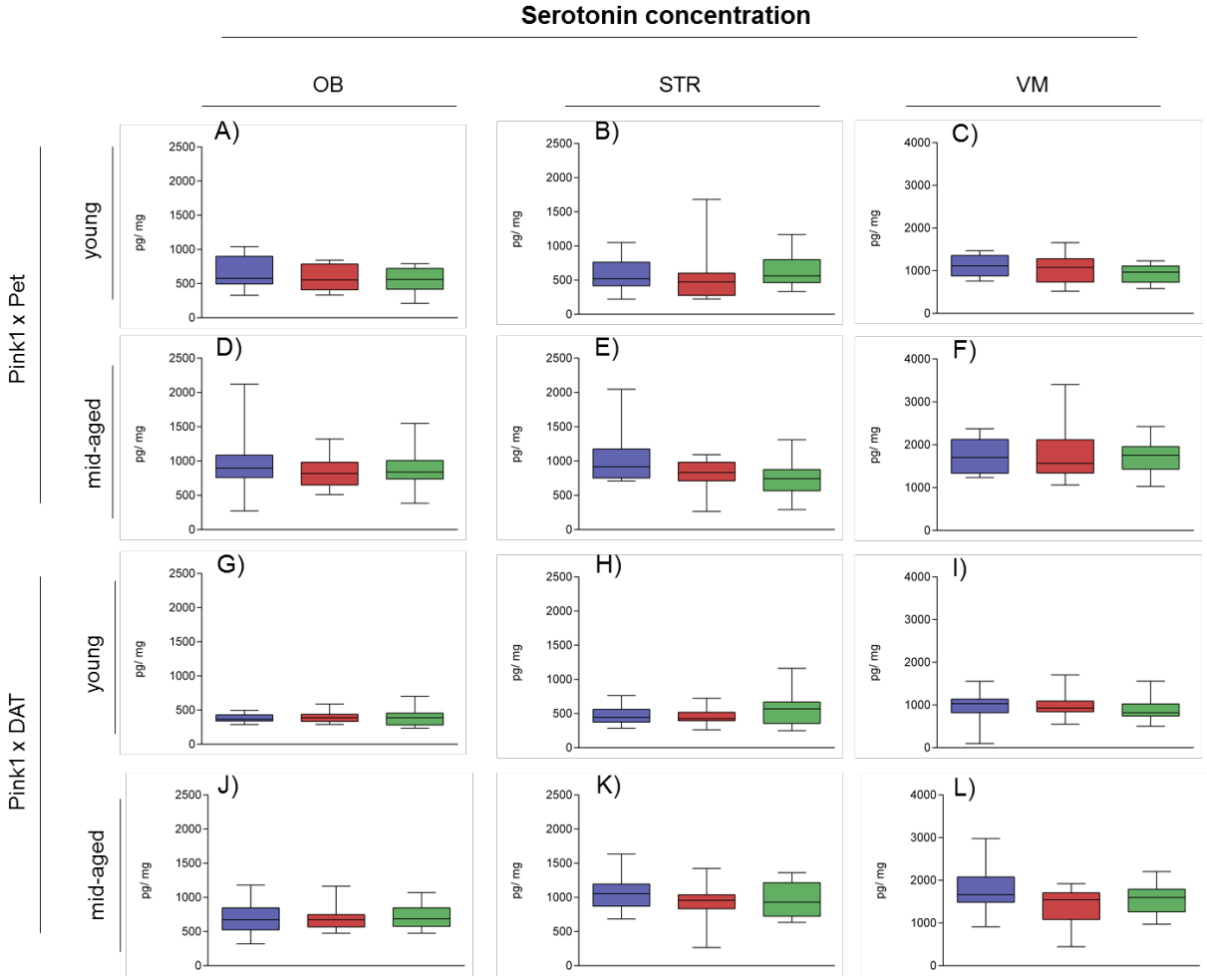

Figure 5.7: Serotonin content in olfactory bulb, striatum and ventral midbrain of Pink1 x Pet and Pink1 x DAT mice at young and mid- age. Three different genotype groups were tested indicated with different colours. All groups were tested in males and females and shown in a grouped manner. Blue = control 1: Cre:Cre+; Pink1:wt/wt. Red = mutant: Cre:Cre+; Pink1:flox/flox. Green = control 2: Cre:Cre-; Pink1:flox/flox. A - F) Pink1 x Pet mice. G - L) Pink1 x DAT mice. Both mouse lines with a young age (A - C, G - I) and mid-age (D - F, J - L) did not exhibit significant differences in the serotonin content in the olfactory bulb (OB), striatum (STR) and ventral midbrain (VM) in the mutant mice compared to control mice. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

5.2.3 Morphological integrity of the serotonergic innervation of the olfactory bulb

In the Pink1_del2/3 mice, a selective reduction of serotonergic innervation of the glomerular layer of the olfactory bulb has been observed (Glasl *et al.*, 2012). Thus, in both mouse lines this innervation was analysed by staining immunohistochemically using an anti-5-HT antibody as marker for this neuronal population. The density of positive neuronal fibers was determined by stereological means at young and middle ages in order to detect whether a) Pink1 acts cell autonomously in the serotonergic neurons with respect to this phenotype and b) Pink1 deficiency in dopaminergic neurons might affect this innervation (methods 4.5 and 4.6).

No significant differences of the serotonergic innervation of the glomerular layer of the OB in young and mid-aged mutant Pink1 x Pet and Pink1 x DAT mice were detected compared to the control groups (Figure 5.8). The statistics of the 5-HT$^+$ innervation is summarized in the ANOVA table A11 and shown in the appendix. A summary of the descriptive analysis of 5-HT$^+$ innervation is listed in the table A12 in the appendix.

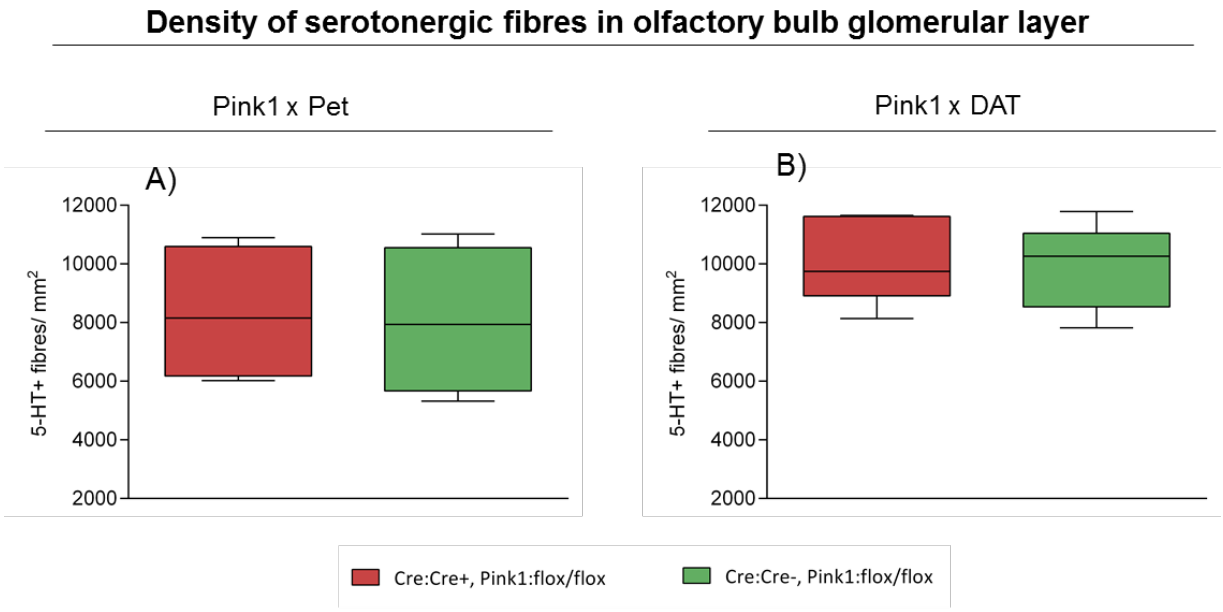

Figure 5.8: Density of serotonergic fibres into the olfactory bulb in Pink1 x Pet and Pink1 x DAT mice at mid-age. Two different genotype groups were tested indicated with different colours. Red = mutant: Cre:Cre+; Pink1:flox/flox. Green = control 2: Cre:Cre-; Pink1:flox/flox. (A) Pink1 x Pet mice. (B) Pink1 x DAT mice. Both mouse lines did not exhibit significant differences in the serotonergic innervation of the glomerular layer of the olfactory bulb. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

To summarize the neuroanatomical and biochemical analysis: Both conditional mouse lines revealed no alterations in either the dopaminergic or the serotonergic system concerning their morphological and/or biochemical integrity. Thus, (i) conditional inducible Pink1 deficiency in the dopaminergic system is not sufficient to induce dopaminergic neurodegeneration, thus cannot overcome any – if present – compensatory mechanisms in place in mice until the age of 14 - 17 months. In addition, (ii) the reduced innervation of the olfactory bulb by serotonergic fibers in the full KO is not based on cell autonomous mechanisms of serotonergic neurons and is not influenced or elicited by Pink1 deficiency in the dopaminergic system.

5.3 Analysis of activity and anxiety-related behaviour

PD patients suffer from motoric impairments, such as a decreased locomotor activity. In addition, neuropsychiatric disturbances like anxiety abnormalities are reported in PD cases (Jankovic, 2008). In order to analyse the influence of a Pink1 deficiency in serotonergic and dopaminergic neurons on locomotion, exploration and anxiety-related behaviour, mice were tested in the OF (Tatem *et al.*, 2014, Gould *et al.*, 2009). For this purpose, mice were placed in an open field arena to record the movement of the mice by infrared sensors (Walsh and Cummins, 1976, Gates *et al.*, 2011). Hereby the ActiMot System was used. The horizontal and vertical locomotor activity of the mice were monitored to gather information concerning rearing frequency, distance travelled, location (methods, figure 4.2).

The OF parameters Distance travelled [cm], Resting time [sec], Permanence time [sec] and Average speed [cm/sec] were examined separately in the centre, periphery and in the whole arena, respectively, over the 20 min time (Permanence time [sec] of whole area equals the 20 min testing time and was equal for all mice). In addition to that, the % Time spent in the centre [%], the % Distance travelled in the centre [%] and the Number of rearing [#] were examined within 5 min intervals and in total, in order to study the behavioural progress over time. Likewise, the Latency to enter in the centre [sec] and the Number of entries in the centre [#] were analysed. In total 32 different OF parameters were investigated.

The statistics of the OF results are summarized in the ANOVA tables A19 – A22 and shown in the appendix. A summary of the descriptive analysis of the parameter: Total distance travelled [cm], Total number of rearing [#], % Time spent in centre [%] and Centre resting time [sec] is listed in the table A23 in the appendix.

5.3.1 Total distance travelled [cm]

In order to analyse locomotion, the parameter Total distance travelled [cm] was analysed. There were no significant differences detectable in the Total distance travelled [cm] between the mutant mice and the control groups in both mouse lines at young and mid-age (figure 5.9).

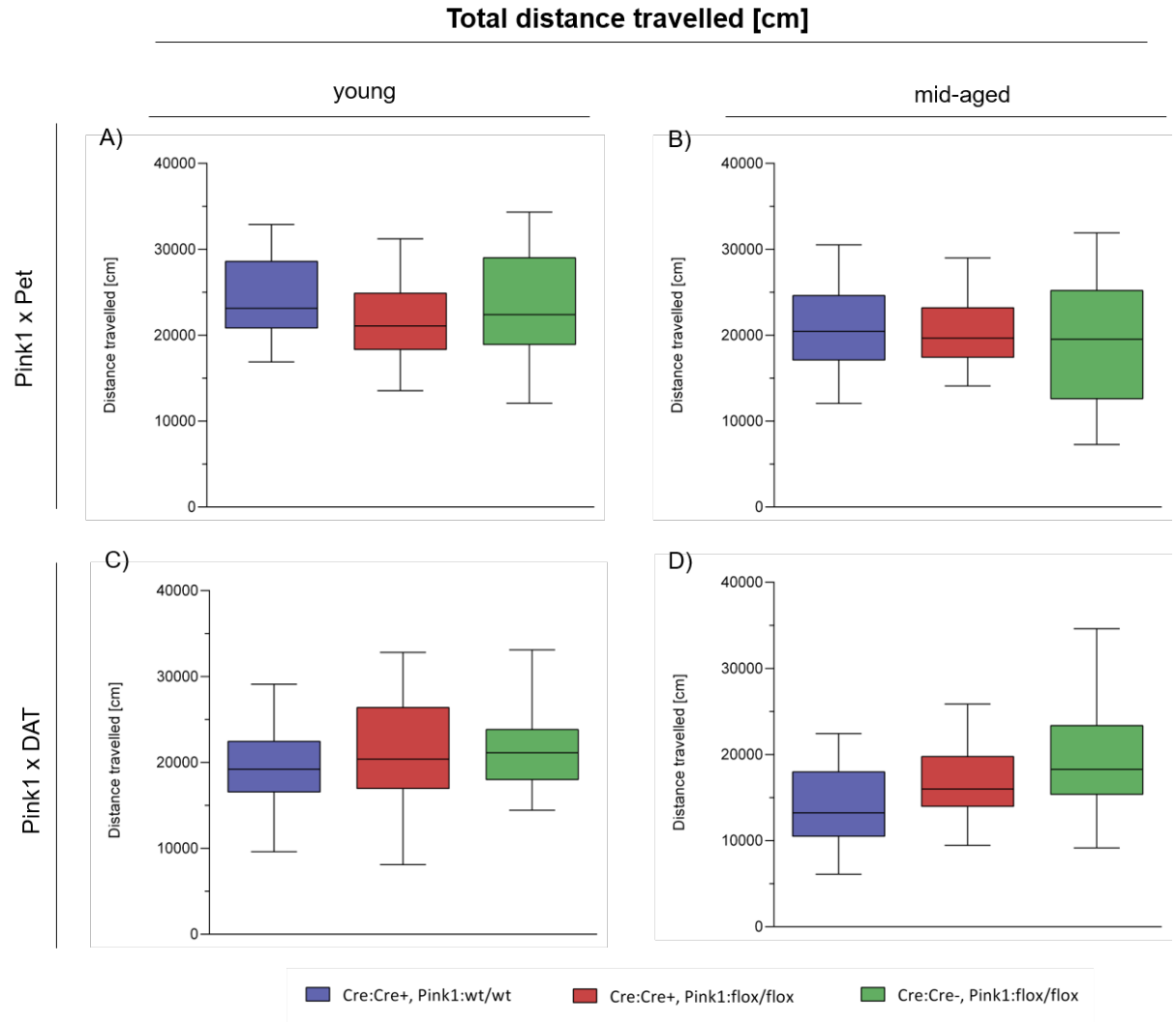

Figure 5.9: Total distance travelled [cm] of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different genotype groups were tested indicated with different colours. All groups were tested in males and females and shown in a grouped manner. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. (A & B) Pink1 x Pet mice. (C & D) Pink1 x DAT mice. Both mouse lines with a young age (A & C) and mid-age (B & D) did not exhibit significant differences in the Total distance travelled [cm] between the mutant and control mice. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

5.3.2 Total number of rearing [#]

In order to analyse the explorative behaviour, the parameter total rearing [#] was measured in the open field. The young and mid-aged mutant mice of both mouse lines did not show significant differences in the Total number of rearing [#] compared to the control groups (figure 5.10).

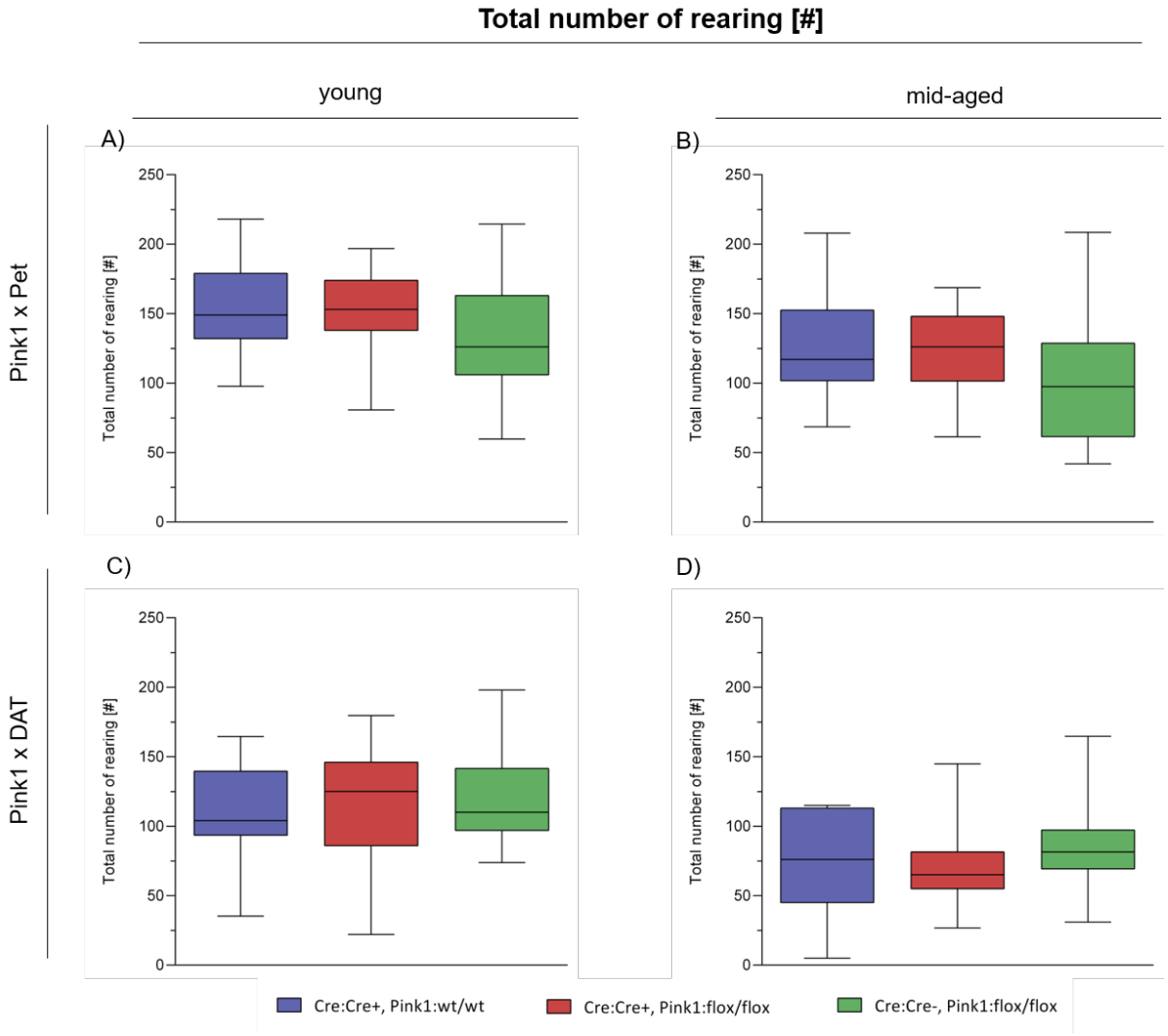

Figure 5.10: Total number of rearing [#] of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different genotype groups were tested indicated with different colours. All groups were tested in males and females and shown in a grouped manner. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. (A & B) Pink1 x Pet mice. (C & D) Pink1 x DAT mice. Both mouse lines with a young age (A & C) and mid-age (B & D) did not exhibit significant differences in the Total number of rearing [#] between the mutant and control mice. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

5.3.3 % Time spent in centre [%]

In order to get information about the anxiety-related behaviour the % Time spent in centre [%] was analysed. The young and mid-aged mutant mice of both mouse lines did not exhibit a significant difference in the % Time spent in the centre [%] compared to both control groups (figure 5.11).

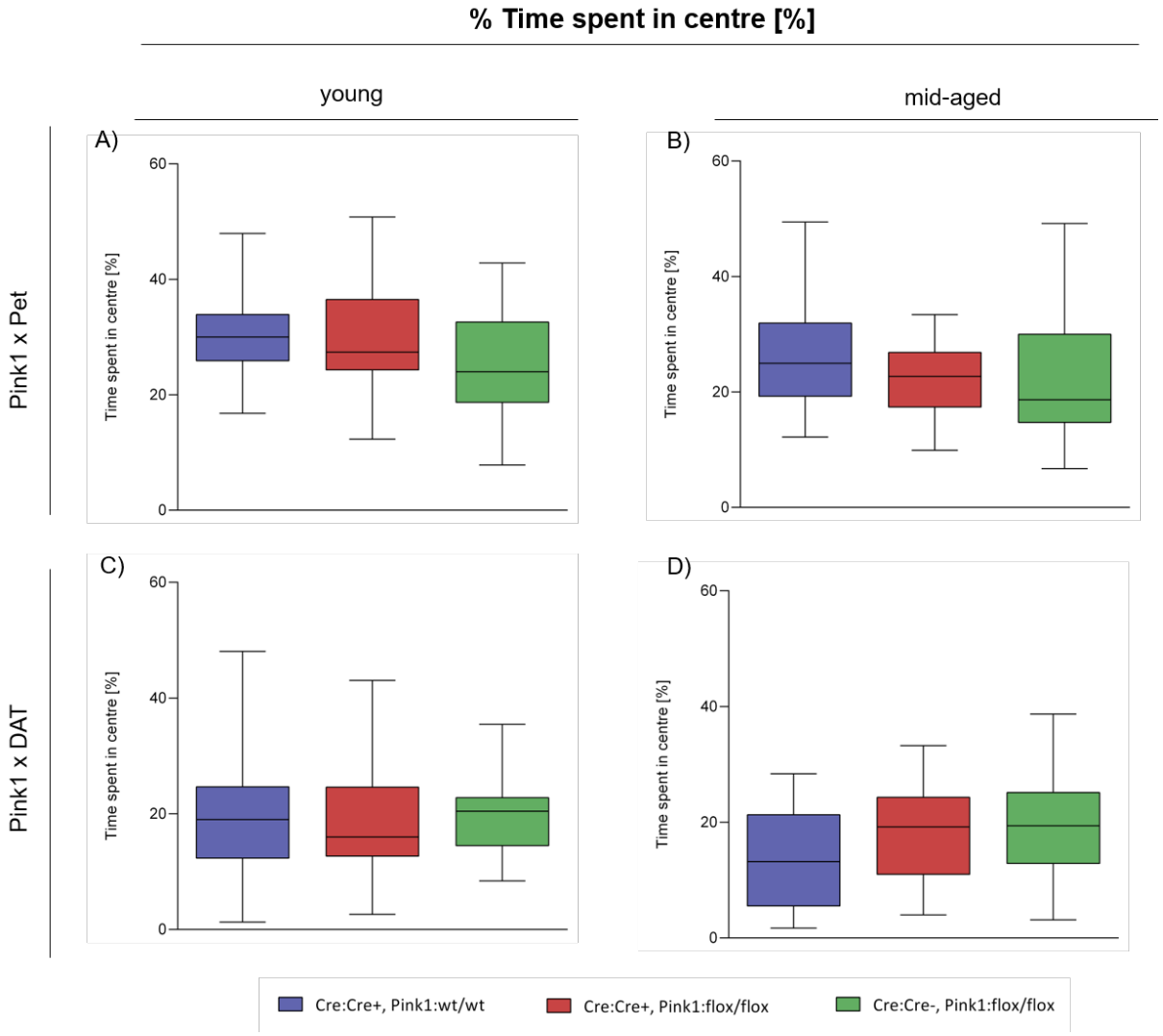

Figure 5.11: % Time spent in centre [%] of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different genotype groups were tested indicated with different colours. All groups were tested in males and females and shown in a grouped manner. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. (A & B) Pink1 x Pet mice. (C & D) Pink1 x DAT mice. Both mouse lines with a young age (A & C) and mid-age (B & D), did not exhibit significant differences in the Time spent in centre [sec] between the mutant and control mice. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

5.3.4 Centre resting time [sec]

In order to get additional information about the anxiety-related behaviour, the Centre resting time [sec] was analysed. The young Pink1 x Pet mutant mice rested on average 46.07 sec (SD = 22.87) in the centre where the mice of the ctrl.1 rested 32.76 sec (SD= 16.02) and the ctrl.2 rested 23.26 sec (SD = 14.49). The mutant Pink1 x Pet mice rested significantly more time in the centre compared to both control groups ($F_{(2,70)}$ = 8.863, mt vs ctrl1: p = 0.031; mt vs ctrl.2, p = 0.0001) (figure 5.12).

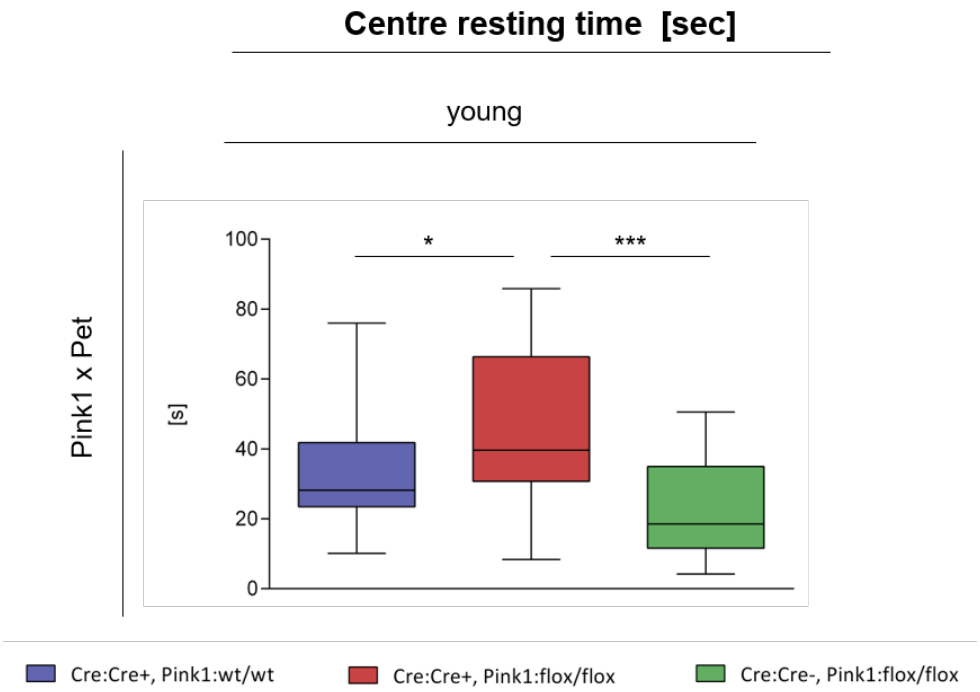

Figure 5.12: Centre resting time [sec] in Pink1 x Pet mice at young age. Three different groups were tested indicated with different colours. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. All groups were tested in males and females and shown in a grouped manner. The young mutant mice rested significantly more time in the centre compared to both controls groups ($F(2,70) = 8.863$, mt vs ctrl.1, $p = 0.031$; mt vs ctrl.2, $p = 0.0001$). The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data. For statistical analysis the multivariant 2-way ANOVA was used followed by the multiple comparison with the post hoc test Bonferroni. p-value ≤ 0.05 = *, p-value ≤ 0.001 = ***.

To summarize the OF testing: The young Pink1 x Pet mutant mice (Cre:Cre+; Pink1:flox/flox) rested significantly more time in the centre [sec] compared to the control groups (Cre:Cre+, Pink1:wt/wt and Cre:Cre-; Pink1:flox/flox). Apart from that, no other parameter, analysed in the open field, was significantly affected in the mutant mice compared to the control groups in the young and mid-aged Pink x Pet and Pink x DAT mice.

5.4 Analysis of basic reflex pathway and sensorimotor gating

The ASR is a stereotyped motor response to an abrupt and intense acoustic stimulus, which is expressed by a contraction of the major muscles (Zhang *et al.*, 2008). When a less intense acoustic stimulus is presented immediately before the acoustic stimulus, the reflex is attenuated. This phenomenon is called PPI of the ASR. PPI is a measure for sensorimotor gating, which reflects the filtering function of irrelevant information (Zoetmulder *et al.*, 2014). ASR and PPI are defined as a biomarker for healthy brain circuitries (Swerdlow *et al.*, 2016). A reduction of striatal dopamine level is associated

with PPI deficits. Likewise, animals with striatal lesions exhibited PPI deficits (Vuillermot *et al.*, 2011). Since PD is characterized by a loss of striatal dopaminergic neurons, the interest was to study the ASR and PPI in the present mouse models of PD (Fearnley and Lees, 1991).

In order to examine the ASR and PPI, animals were placed in a sound attenuated box in which the animals were exposed to random stimuli at different sound levels. The resulting muscular reflex reaction was measured using a startle platform (methods figure 4.3). In order to examine the ASR, stimuli with a sound pressure of 70 dB, 80 dB, 85 dB, 90 dB, 100 dB, 110 dB and 120 dB were used. In order to avoid body weight effects, all data from ASR were corrected for body weight. The background movement of the mice was detected by the null stimulus (NS). The PPI was studied by a stimulus with 110 dB preceded by different prepulses with the sound pressure levels of 67 dB, 69 dB, 73 dB and 81 dB.

The statistics of the ASR and PPI results are summarized in the ANOVA tables A24 & A25 and shown in the appendix. Summaries of the descriptive analysis of the ASR and PPI are listed in the tables A26 - A29 in the appendix.

5.4.1 Acoustic startle reflex (ASR)

In order to analyse the integrity of the brain circuitry of the reflex pathway from auditory nerve to the reticulospinal tract the ASR was analysed (Zhang *et al.*, 2008). Mid-aged male mutant Pink1 x Pet mice startled on average 13.856 [arbitrary units (a.u.)] (SD = 4.725) whereas the mice of the ctrl.1 startled 7.432 a.u. (SD = 2.646) and the ctrl.2 startled 8.835 a.u. (SD = 2.279). The male mutant Pink1 x Pet mice startled significantly more at a sound pressure level of 120 dB compared to both male control groups ($F_{(2,31)} = 9.819$, mt vs ctrl1: $p = 0.001$; mt vs ctrl.2, $p = 0.013$) (figure 5.13).

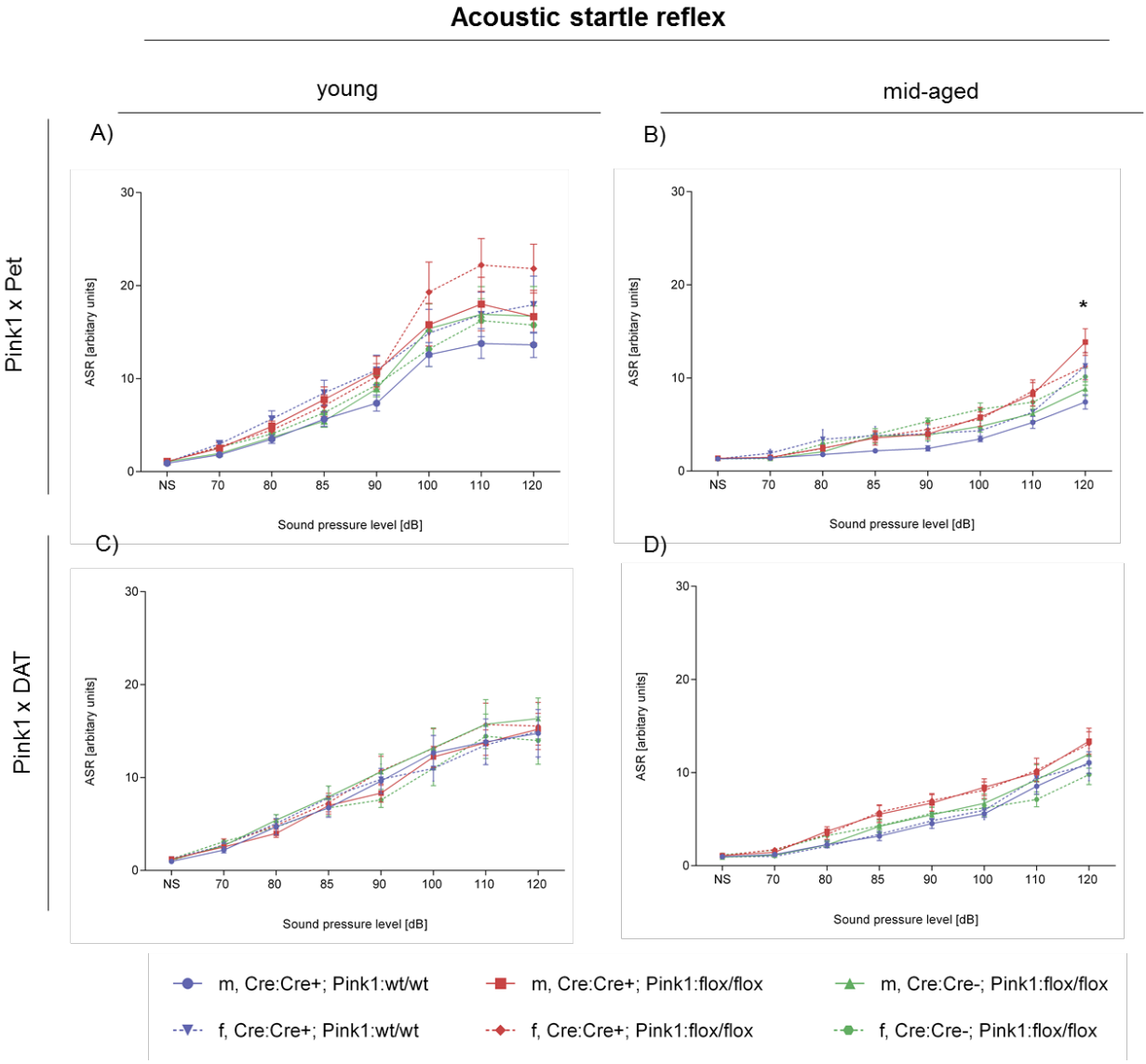

Figure 5.13: Acoustic startle reflex Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different groups were tested indicated with different colours. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. All groups were tested in males and females, indicated by the letters "m" and "f".The ordinates represent the ASR, which is the amount of movement (reflex) due to an acoustic stimulus, expressed in arbitrary units. The abscissa represent the stimuli steps with increasing sound pressure level starting from 70 dB up to 120 dB. The null stimulus was measured to identify the background movement without a stimulus. (A & B) Pink1 x Pet mice. (C & D) Pink1 x DAT mice. The young (A & C) mutant mice did not show significant differences in acoustic startle reflex compared to the control groups. (B) The mid-aged male mutant mice startled significantly more compared to both control groups at the stimulus of 120 dB ($F_{(2,29)}$ = mt vs ctrl1: p = 0.001; mt vs ctrl.2, p = 0.013). (D) The mid-aged mutant mice did not show significant differences in acoustic startle reflex compared to the control groups. For statistical analysis, data were analysed with multivariant 2-way ANOVA testing followed by the post hoc test with Bonferroni, $p \leq 0.05$ = *. The data of different mice per group are summarized and presented with +/- S.E.M.

5.4.2 Prepulse inhibition (PPI)

In order to analyse the filtering mechanism of the brain, the PPI was analysed. The mutant mice of the tested mouse lines did not exhibit significant differences in PPI compared to both control groups (figure 5.14).

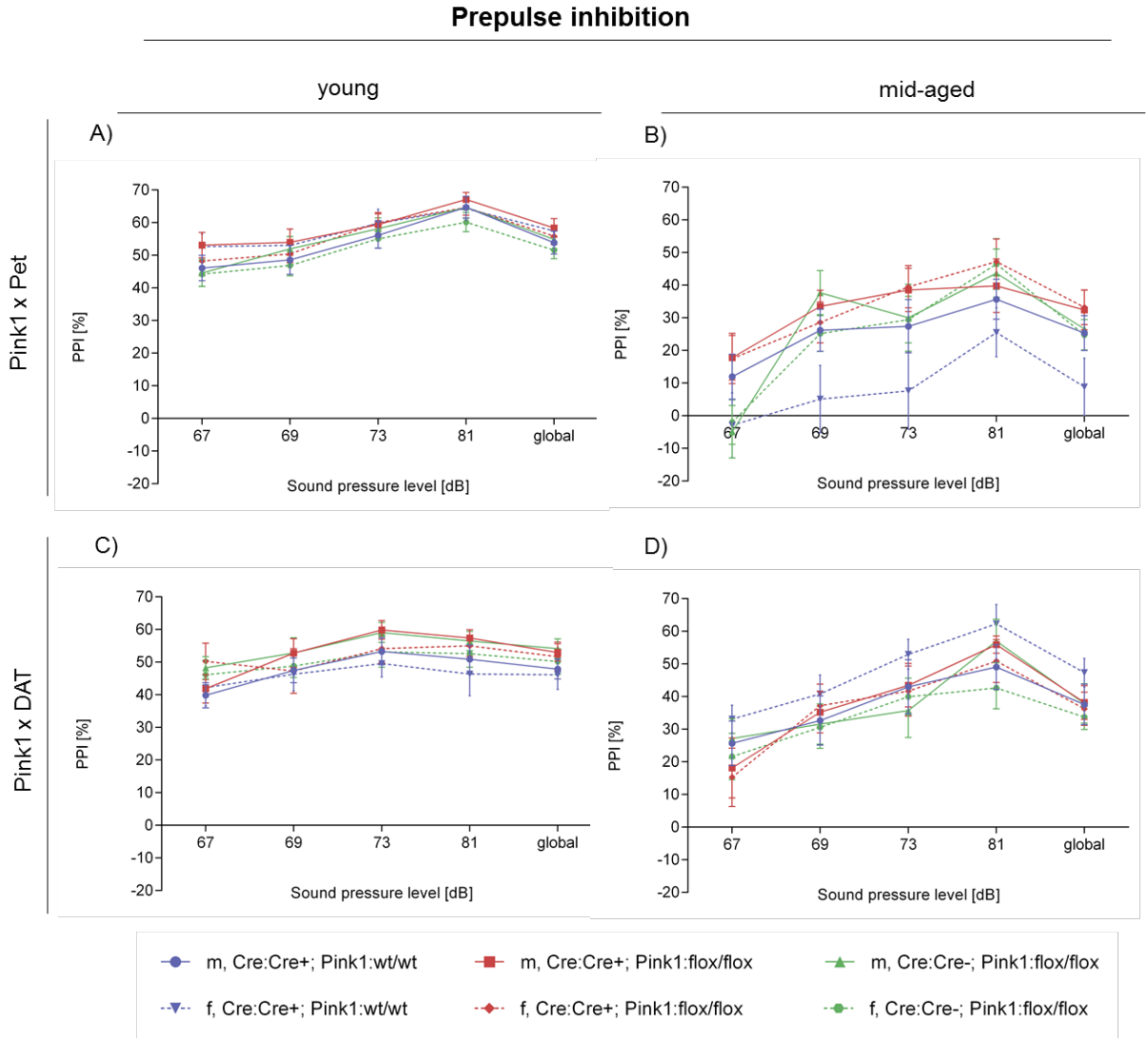

Figure 5.14: Prepulse inhibition of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different groups were tested indicated with different colours. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. All groups were tested in males and females, indicated by the letters "m" and "f". The ordinates represent the PPI, which is the percentage reduction of the startle reflex due to pre-stimuli before the actual stimulus with a sound pressure of 110 dB. The abscissa represents the different pre-stimuli from 67 dB up to 81 dB. The global analysis shows the mean of the PPI over the different pre stimuli. (A & B) Pink1 x Pet (C & D) Pink1 x DAT mice. The young (A & C) and mid-aged (B & D) mutant mice did not show significant differences in prepulse inhibition compared to the control groups. The data of different mice per group are summarized and presented with +/- S.E.M.

To summarize the ASR and PPI testing: The male mutant mid-aged Pink1 x Pet mice startle significantly more at a sound pressure level of 120 dB compared to both male control groups. Apart from that, mutant mice did not show significant differences compared to the control groups in ASR and PPI.

5.5 Analysis of the gait

Apart from the typical motor symptoms like bradykinesia, rest tremor or muscle rigidity PD patients exhibit gait disturbances. Gait impairments develop already in the very early stages of the disease, which usually becomes more severe at later stages (Erro and Stamelou, 2017). In order to research the influence of Pink1 deficiency on serotonergic and dopaminergic neurons on gait at different ages, mice were analysed with the CatWalk XT system. Animals were placed on a glass plate where free walking of the mice was enabled. A camera underneath the glass plate recorded the walk (Holter *et al.*, 2015) (methods, figure 5.4).

An array of different parameters were analysed including the temporal sequence during walking, the comparison of paw placements, the interlimb coordination and the individual paw. In total 35 different parameters were considered for the analysis of the single and grouped paws. A description of the CW parameters is listed in table A30 in the appendix. Whereas the main findings of this analysis are depicted below, supplementary information is given in the appendix: the statistics of the CW results are summarized in the ANOVA tables A31 - 46 and shown in the appendix. A summary of the descriptive analysis of Pink1 x Pet mice of the selected CW parameter: Minimum intensity [pixel] of LF paws, % Max contact at [%] of left front (LF) paws and front paws (FP), and phase dispersion of LF-RF (left front - right front) paws is listed in the table A47 in the appendix. A summary of the descriptive analysis of Pink1 x DAT mice of the selected CW parameter: Stance duration [sec] of right hind (RH) paws and hind paws (HP), Body speed [cm/sec] of left hind (LH) paws and HP, Swing speed [cm/sec] of LH paws and HP and Base of support of HP is listed in the tables A48 - A49 in the appendix.

5.5.1 Gait in Pink1 x Pet mice

Minimum intensity [pixel], LF paws: The LF paws of the young male Pink1 x Pet mutant mice were placed on the glass plate with a minimum intensity of 28.74 pixel (SD = 0.38). The pixel was recorded in a range from 0 to a maximum of 255 pixel. Hence, lower numbers refer to a lower intensity of placed paws on the glass plate. The young male ctrl.1 mice placed the LF paw with a minimum intensity of 29.19 pixel (SD = 0.47) on the glass plate and the young male ctrl.2 mice reached a minimum intensity of the LF paws of 29.28 [pixel] (SD = 0.39). The young male Pink1 x Pet mutant mice showed a tendency to a lower minimum intensity compared to ctrl.1 ($F_{(2,27)} = 4.455$, $p = 0.032$)

and exhibited significantly lower minimum intensities of the LF paws in comparison to the ctrl.2 ($F_{(2,27)} = 4.455$, $p = 0.062$) (Figure 5.15, A).

% Maximum contact at [%], LF paws: In addition, young female Pink1 x Pet mutant mice established a maximum contact of the LF paws with the glass plate after 40.02 % (SD = 4.93) of the total glass contact time. The LF paws of the young female ctrl.1 mice reached the glass contact after 32.81% (SD = 7.08) and ctrl.2 mice after 33.24% (SD = 5.92) of the total glass contact time. The young female Pink1 x Pet mutant mice required significantly more percentage time in order to reach the maximum contact of the LF paws with the glass plate in comparison to the both control groups ($F_{(2,35)} = 4.774$, mt vs ctrl1: $p = 0.023$; mt vs ctrl.2, $p = 0.035$) (Figure 5.15, B).
The same effect was observed in the mid-aged female Pink1 x Pet mice, where the LF paws of the mutant mice took significantly more percentage time in order to reach the maximum contact of the glass plate compared to both control groups ($F_{(2,32)} = 5.151$, mt vs ctrl1: $p = 0.015$; mt vs ctrl.2, $p = 0.043$). Here, the LF paws of the young female Pink1 x Pet mutant mice established a maximum contact with the glass plate after 42.37% (SD = 3.81) of the total glass contact time. The LF paws of the young female ctrl.1 mice reached the glass contact after 34.85% (SD = 7.39) and ctrl.2 mice after 36.02% (SD = 5.44) of the total glass contact time (Figure 5.15, C).

% Maximum contact at [%], FP paws: Next to the LF paws, a similar effect occurred in the FP of the mid-aged female Pink1 x Pet mice, where the mutant mice placed the FP after 45.66% (SD = 4.02) of the total contact time with the glass plate at a maximum contact. The FP of the mid-aged female ctrl.1 mice reached the maximum contact after 40.69% (SD = 5.00) and ctrl.2 mice after 38.57% (SD = 4.85) of the total glass contact time. The mid-aged female Pink1 x Pet mutant showed a tendency to higher percentage time in comparison to the ctrl.2 mice and needed significantly more percentage time to place the FP at maximum contact in comparison to the ctrl.1 mice ($F_{(2.32)} = 6.639$, mt vs ctrl1: $p = 0.056$; mt vs ctrl.2, $p = 0.003$) (Figure 5.15, D).

Phase dispersion [%], LF-RF: Mid-aged female Pink1 x Pet mutant mice exhibited an impairment in the temporal relationship between the placement of the LF and RF paws (Phase dispersion %) compared to both control groups ($F_{(2.32)} = 8.358$, mt vs ctrl1: $p = 0.002$; mt vs ctrl.2, $p = 0.005$). A phase dispersion of 50.00% describes an optimal temporal relationship of two paws (Kloos *et al.*, 2005). Both control groups of the mid-

aged female Pink1 x Pet mice reached a near optimum phase dispersion (ctrl.1: 49.28% (SD = 2.26) and ctrl.2: 49.67% (SD = 2.26)) in contrast to the mutant mice (52.77%, SD = 2.57). The mid-aged female mutant Pink1 x Pet mice exhibited significantly higher percentages in phase dispersion of LF-RF paws ($F_{(2,32)} = 8.358$, mt vs ctrl.1: $p = 0.002$; mt vs ctrl.2, $p = 0.005$) (Figure 5.15, E).

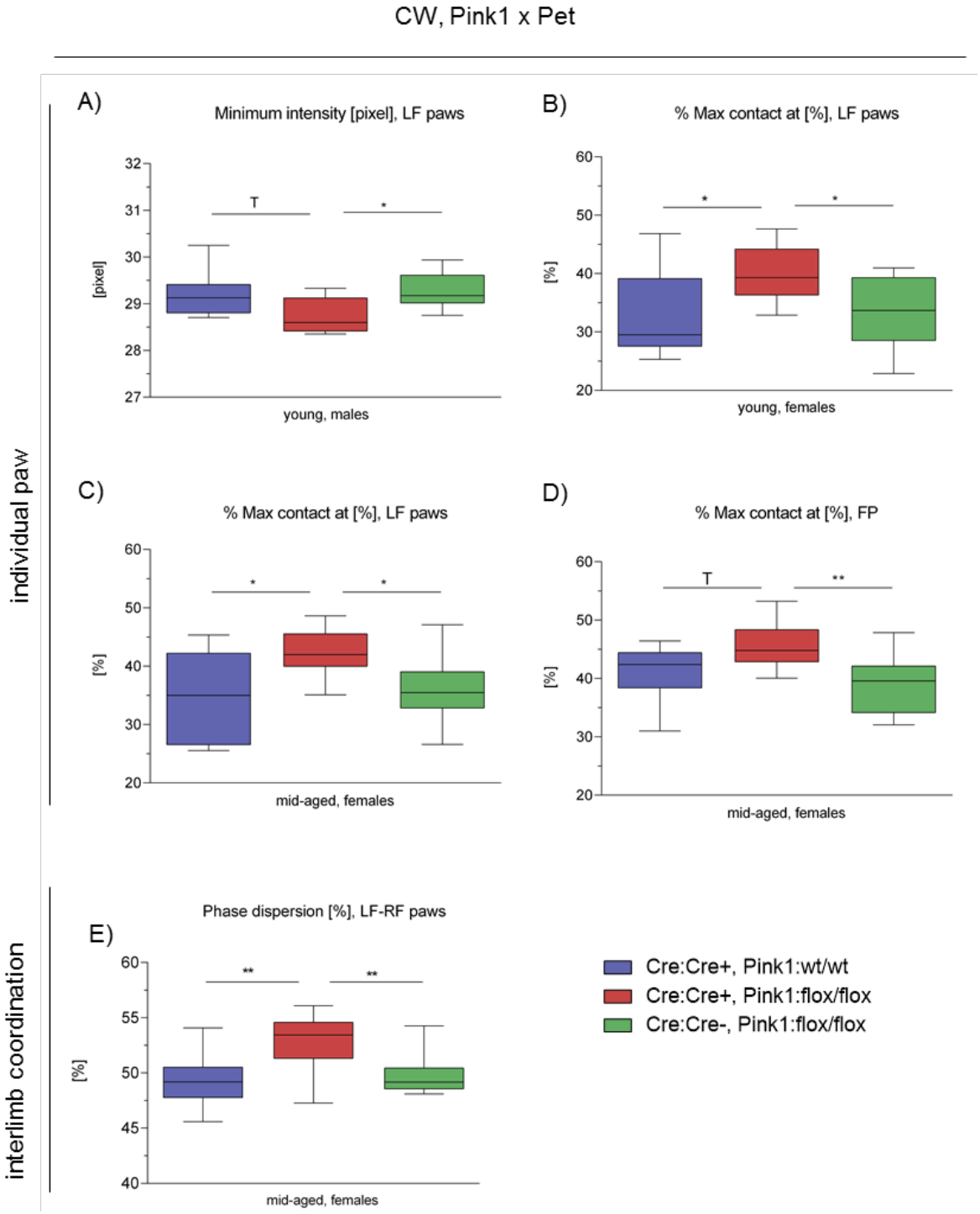

Figure 5.15: Affected gait parameters Pink1 x Pet mice at young and mid-age. Three different groups were tested indicated with different colours. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. Males and females were tested separately. LF = left front paws, RF = right front paws, FP = front paws. A) Young male mice showed a

tendency to place the LF paws with a lower minimum intensity as compared to the ctrl.1 (F (2,27) = 4.455, p = 0.032) and exhibited significant lower minimum intensities of the LF paws in comparison to the ctrl.2 (F (2,27) = 4.455, p = 0.062). B) Young and C) mid-aged female mutant mice required significantly more percentage time in order to establish a maximum contact with the LF paws on the glass plate compared to both control groups (young: (F (2,35) = 4.774, mt vs ctrl1: p = 0.023; mt vs ctrl.2, p = 0.035; mid-aged: (F (2,32) = 5.151, mt vs ctrl1: p = 0.015; mt vs ctrl.2, p = 0.043)). D) Mid-aged female mutant mice showed a tendency to a higher percentage time of establishing the maximum contact of the FP with the glass plate compared to the ctrl.1 (F (2.32) = 6.639, p = 0.056). However, mid-aged female mutant mice established a significant higher percentage maximum contact time compared to the ctrl.2 (F (2.32) = 6.639, p = 0.003). E) Mid-aged female mutant mice established significantly higher percentages of phase dispersion compared to both control groups (F (2.32) = 8.358, mt vs ctrl1: p = 0.002; mt vs ctrl.2, p = 0.005). For statistical analysis, data were analysed with multivariant 2-way ANOVA testing followed by the post hoc test with Bonferroni, $p \leq 0.05$ = *. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

5.5.2 Gait in Pink1 x DAT mice

Stance duration [sec], RH paws: Stance duration is the duration of paw contact with the glass plate during one paw placement. The RH paws of the young Pink1 x DAT mutant mice exhibited a stance duration of 0.11 sec (SD = 0.01), whereas the ctrl.1 mice required 0.10 sec (SD = 0.02) and the ctrl.2 mice required 0.10 sec (SD = 0.02). The RH paws of the young Pink1 x DAT mutant mice exhibited a significantly higher stance duration as compared to both control groups ($F_{(2,66)}$ = 4.282, mt vs ctrl1: p = 0.028; mt vs ctrl.2: p = 0.038) (Figure 5.16, A).

Stance duration [sec], HP: A similar effect was observed in the stance duration of the HP of the young Pink1 x DAT mutant mice. Here, the mutant mice exhibited a stance duration of 0.11 sec (SD = 0.01), the ctrl.1 mice of 0.10 sec (SD = 0.02) and the ctrl.2 mice of 0.11 sec (SD = 0.02). The young mutant mice exhibited a significantly higher stance duration of the HP compared to the ctrl.1 mice ($F_{(2,66)}$ = 3.711, p = 0.033) and showed a tendency to higher stance duration of the HP to the ctrl.2 mice ($F_{(2,66)}$ = 3.711, p = 0.090) (Figure 5.16, B).

Body speed [cm/sec], LH paws: The speed of the body from one initial contact of the LH paw to the next initial contact of the young Pink1 x DAT mutant mice was 24.23 cm/sec (SD = 4.57). The young ctrl.1 mice showed a body speed of the LH paw of 27.55 cm/sec (SD = 4.63) and the ctrl.2 mice of 27.52 cm/sec (SD = 5.29). The young mutant Pink1 x DAT mice showed a tendency to a slower body speed of the LH paw as compared to both control groups ($F_{(2.66)}$ = 3.515, mt vs ctrl1: p = 0.068; mt vs ctrl.2, p = 0.064) (Figure 5.16, C).

Body speed [cm/sec], HP: The same effect was observed in the body speed of the HP, where young Pink1 x DAT mutant mice showed a tendency to a slower body speed compared to both control groups ($F_{(2.66)} = 3.288$, mt vs ctrl1: $p = 0.092$; mt vs ctrl.2, $p = 0.070$). The young mutant mice exhibited a body speed of HP of 24.27 cm/sec (SD =4.56), the ctrl.1 mice of 27.42 cm/sec (SD = 4.55) and the ctrl.2 mice of 27.51 cm/sec (SD = 5.37) (Figure 5.16, D).

Swing speed [cm/sec], LH paws: The young Pink1 x DAT mutant mice swung the LH paws during the time without with the glass plate (swing phase) with a speed of 55.91 cm/sec (SD = 10.39) where the LH paws of the ctrl.1 mice swung with a speed of 64.19 cm/sec (SD = 10.41) and of ctrl.2 mice with a speed of 64.24 cm/sec (SD = 11.61). The young Pink1 x DAT mutant mice swung the LH paws with a significantly slower speed compared to both control groups ($F_{(2.66)} = 6.095$, mt vs ctrl.1: $p = 0.007$; mt vs ctrl.2: $p = 0.006$) (Figure 5.16, E).

Swing speed [cm/sec], HP: The same effect was observed in the swing speed of the HP of the young Pink1 x DAT mice. The young Pink1 x DAT mutant mice swung the HP with a speed of 55.60 cm/sec (SD = 10.37), the ctrl.1 mice with a speed of 62.71 cm/sec (SD = 8.62) and the ctrl.2 mice with a speed of 62.25 cm/sec (SD = 11.15). The young Pink x DAT mutant mice swung the HP during the swing phase significantly slower compared to both control groups ($F_{(2.66)} = 4.541$, mt vs ctrl.1: $p = 0.017$; mt vs ctrl.2, $p = 0.024$) (Figure 5.16, F).

Base of support [cm], HP: The mid-aged male Pink1 x DAT mutant mice displayed a distance between the HP of 2.81 cm (SD = 0.21). In comparison the ctrl.1 mice showed HP distances of 3.19 cm (SD = 0.22) and ctrl.2 mice of 3.07 cm (SD = 0.20). The mid-aged male mutant mice put the HP significantly closer together compared to both control groups ($F_{(2, 28)} = 8.405$, mt vs ctrl.1: $p = 0.001$; mt vs ctrl.2, $p = 0.035$) (Figure 5.16, G & 5.17).

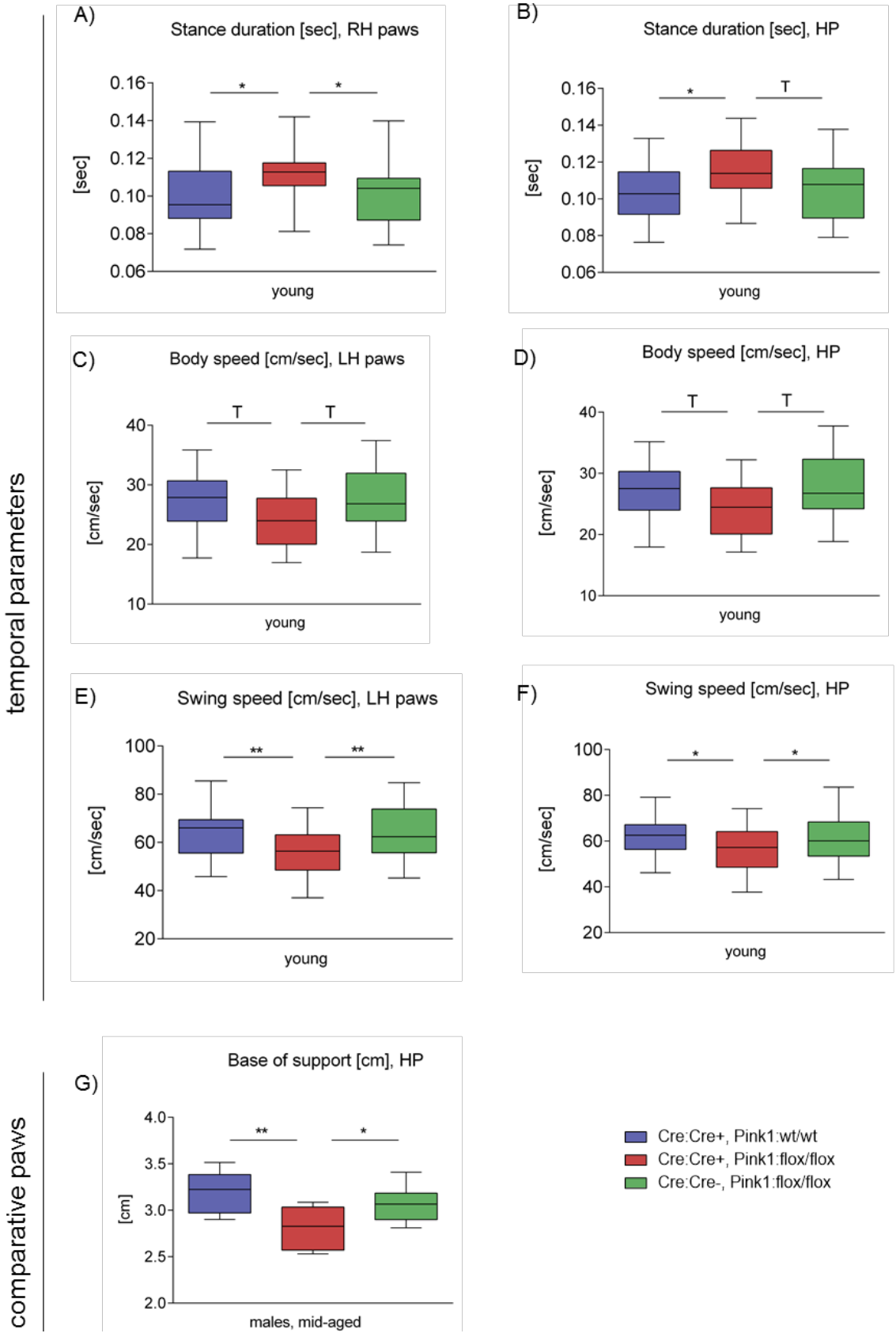

Figure 5.16: Affected gait parameters in Pink1 x Pet mice at young and mid-age. Three different groups were tested indicated with different colours. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. Males and females were tested separately and partially shown in a grouped manner. RH = right hind paws, HP = hind paws, LH = left

hind paws. A) Young mutant mice exhibited a significantly higher stance duration of the RH paws compared to both control groups (F (2,66) = 4.282, mt vs ctrl1: p = 0.028; mt vs ctrl.2: p = 0.038). B) The young mutant mice showed a significantly higher stance duration of the HP compared to the ctrl.1 and a tendency to higher stance duration compared to the ctrl.2 (F (2,66) = 3.711; mt vs ctrl.1: p = 0.033; mt vs ctrl.2: p = 0.090). E & F) The young mutant mice swung the LH and HP significantly slower compared to both control groups (LH: F (2,66) = 6.095, mt vs ctrl.1: p = 0.007; mt vs ctrl.2: p = 0.006; HP: F (2,66) = 4.541, mt vs ctrl.1: p = 0.017; mt vs ctrl.2: p = 0.024). G & H) The young mutant mice showed a tendency to a slower body speed with the LH paws and HP compared to both control groups (LH: F (2,66) = 3.515, mt vs ctrl.1: p = 0.068; mt vs ctrl.2: p = 0.064; HP: F (2,66) = 3.288, mt vs ctrl.1: p = 0.092; mt vs ctrl.2: p = 0.070). The mid-aged male mutant mice put the HP significantly closer together compared to control groups (F (2, 28) = 8.405, mt vs ctrl.1: p = 0.001; mt vs ctrl.2, p = 0.035). For statistical analysis, data were analysed with the 2-way ANOVA testing followed by the post hoc test with Bonferroni, $p \leq 0.05$ = *. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

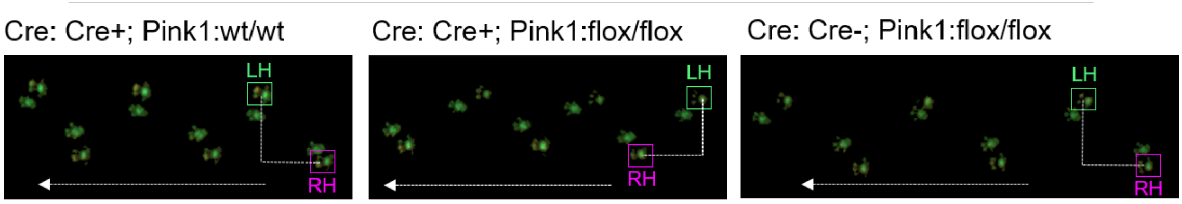

Figure 5.17: Exemplified print view of Pink1 x DAT mice at mid-age. ctrl.1: Cre:Cre+; Pink1:wt/wt. mt: Cre:Cre+; Pink1:flox/flox. ctrl.2: Cre:Cre-; Pink1:flox/flox. The running direction goes from the right side to the left side, indicated by the white arrow. Each example shows two step cycles of the left hind and right hind paw. The dotted lines indicate the base of support, which is the distance [cm] of the both hind paws. The vertical dotted line of the mutant mice Cre:Cre+, Pink1:flox/flox is shorter compared to that one of the both control groups.

To summarize the CW testing: Both tested mouse lines Pink1 x Pet and Pink1 x DAT displayed selective affected parameters due to the Pink1 deficiency in serotonergic and dopaminergic neurons.

The young male Pink1 x Pet mutant mice established partially a lower minimum intensity of the LF paw compared to the control groups. In addition, young female Pink1 x Pet mutant mice displayed higher percentage time until the LF paws reached the maximum contact compared to the control groups. This phenotype was also present in the mid-aged female Pink1 x Pet mutant mice. In addition, the FP paws of the mid-aged female Pink1 x Pet mutant mice needed more percentage time compared to the control groups in order to reach the maximum contact with the glass plate. Apart from the individual paw-related parameters, mid-aged female Pink x Pet mutant mice showed higher percentages in phase dispersion of the LF-RF paws, which represents the interlimb coordination.

The young Pink1 x DAT mutant mice took more time in the stance duration of the RH paws and HP, exhibited a slower swing speed of the LH paws and HP and showed a decreased body speed of the LH paws and HP in comparison to both control groups. Apart from the temporal parameters, mid-aged Pink1 x DAT mutant mice showed a

decreased base of support in comparison to both control groups, which represents the comparative paws.

5.6 Analysis of the olfactory function

Olfactory impairments are dominant in more than 90% of PD patients (Schapira *et al.*, 2017). Male Pink1_del2/3 mice developed olfactory dysfunction due to the Pinkl1 deficiency (Glasl *et al.*, 2012). In order to dissect the influence of the distinct neurotransmitter systems on the olfactory dysfunction with Pink1 deficiency, Pink1 x Pet and Pink1 x DAT mice were tested for olfactory function. Therefore, mice were tested using two different approaches: testing of olfactory discrimination of binary mixtures and testing of olfactory sensitivity threshold. Before that, mice were trained to the odorant [S+] by rewarding them with chocolate pellets. During the testing of binary mixture discrimination, mice needed to choose the odorant [S+], which was diluted with the odorant [S-] at different concentration levels of 100%, 70%, 55%, 53% and 51%, for example a mixture of 51% includes 51% of the odorant [S+] and 49% of the odorant [S-]. The percentage of correct choices was recorded. Afterwards, mice were exposed to the odorant, which was diluted in a stepwise manner and recorded until mice were able to detect the diluted odorant [S+] (methods figure 4.7). Since only male Pink1_del2/3 mice were previously tested on olfactory performance, only male Pink1 x Pet and Pink1 x DAT mice were considered for olfactory testing. The statistics of the olfactory testing results are summarized in the ANOVA table A50 and shown in the appendix. A summary of the descriptive analysis of the olfaction tests are listed in the table A51 in the appendix.

5.6.1 Analysis of binary mixture discrimination

In order to analyse the olfactory fine discrimination, mice were tested on a binary discrimination task. The mutant mice of the tested mouse lines did not exhibit significant differences in the percentage of correct choices of the odorant [S+] compared to both control groups (figure 5.18).

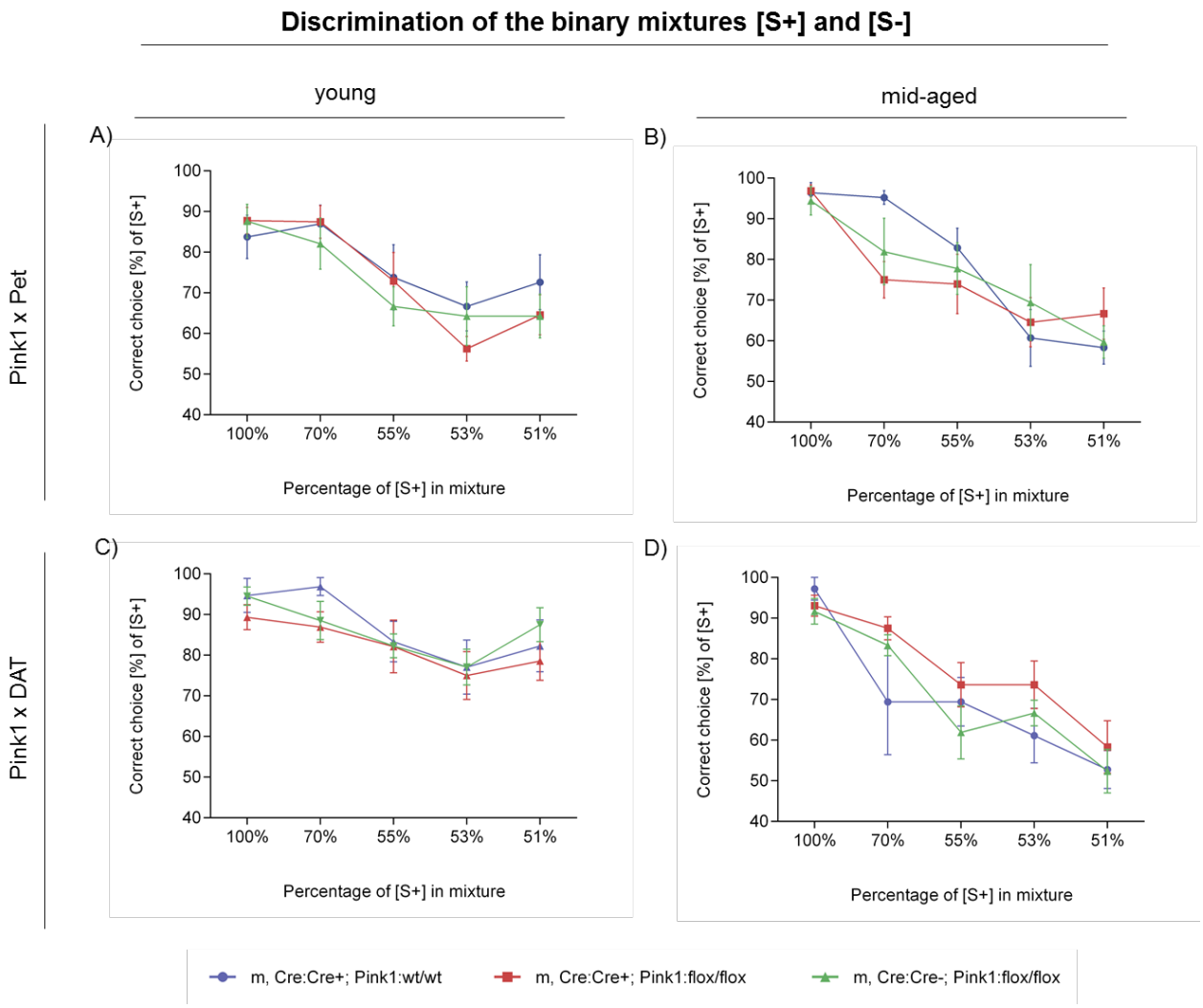

Figure 5.18: Binary mixtures testing of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different groups were tested indicated with different colours. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. Only male mice were tested, indicated by the letter "m" in the legend. The original 10% concentrated odorant [S+] and [S-] were labelled as 100% concentrated. All odorants percentages stand for volume percent (v/v). The ordinates represent the percentage of successful discrimination of the odorant [S+] that was in mixture with the odorant [S-]. The abscissa represents the different concentrations 100%, 70%, 55%, 53% and 51% (v/v) of the odorant [S+] in mixture with the odorant [S-]. (A & B) Pink1 x Pet mice. (C & D) Pink1 x DAT mice. Both mouse lines with a young age (A & C) and mid-age (B & D), did not exhibit significant differences in discrimination of the odorant [S+] in a mixture with the odorant [S-].The data of different mice per group are presented with +/- S.E.M.

5.6.2 Analysis of olfactory sensitivity

In order to analyse the olfactory sensitivity, the threshold of diluted odorant [S+] of the mice was detected. The mutant mice of the tested mouse lines did not exhibit significant differences in the sensitivity threshold of the odorant [S+] compared to both control groups (figure 5.19).

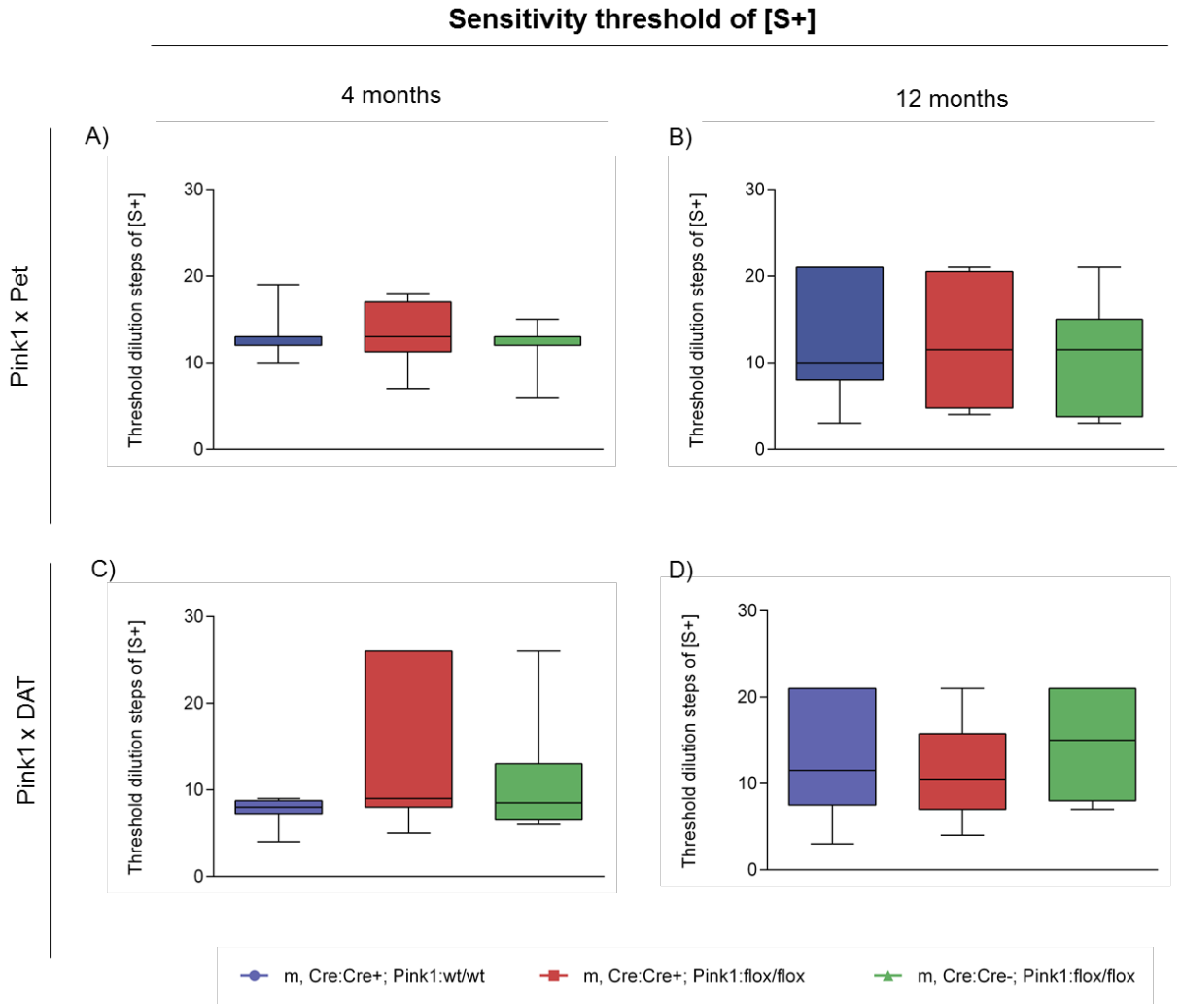

Figure 5.19: Testing of olfactory sensitivity threshold of Pink1 x Pet and Pink1 x DAT mice at young and mid-age. Three different groups were tested indicated with different colours. Blue = ctrl.1: Cre:Cre+; Pink1:wt/wt. Red = mt: Cre:Cre+; Pink1:flox/flox. Green = ctrl.2: Cre:Cre-; Pink1:flox/flox. Only male mice were tested, indicated by the letter "m" in the legend. The ordinates represent the threshold of dilution steps of the odorant [S+] until which mice were able to detect the diluted odorant [S+]. (A & B) Pink1 x Pet mice. (C & D) Pink1 x DAT mice. Both mouse lines with a young age (A & C) and mid-age (B & D), did not exhibit significant differences in threshold of dilution steps of the odorant [S+]. The data are shown as whisker plots with the median as the horizontal line in a box, which demonstrates 50% of the data surrounded by whiskers containing the central 95% of the data.

To summarize the olfactory testing: The young and mid-aged Pink1 x Pet and Pink1 x DAT mutant mice did not exhibit significant differences in the olfactory binary discrimination and sensitivity threshold of the odorant [S+] compared to both control groups.

5.6 Summary

The Pink1 x Pet and Pink1 x DAT mouse lines did not show PD-related neuropathological alteration due to the deficiency of Pink1 in serotonergic and dopaminergic neurons. Neither a neurodegeneration of dopaminergic neurons in the SNC or an impaired serotonergic innervation into the olfactory bulb was observed in the mutant mice. Furthermore, no changes were observed in the content of dopamine and serotonin and its metabolites in different brain tissues. Equally, the analysed mice did not reveal olfactory impairments. Young and mid-aged (male) mutant Pink1 x Pet mice exhibited small genotype effects in the OF testing and in the ASR. In addition, Pink1 x Pet mutant mice showed PD-related gait phenotypes, mainly the left front paw was affected. Also, young and old Pink1 x DAT mice displayed PD-related gait impairments due to affected hind paws. Taken together, Pink1 x Pet and Pink1 x DAT mice exhibited slight PD-related non-motor symptoms, which were partially sex related (summarized in figure 5.20).

Feature	Specification		Pink x Pet, young	age [months]	Pink1 x Pet, mid-aged	age [months]	Pink1 x DAT, young	age [months]	Pink1 x DAT, mid-aged	age [months]
Neuropathology	number of DA neurons in SNC		→	3.7	→	19.7	→	4.4	→	18
	neurotransmitter content in OB, STR and VM		→		→		→		→	
	serotonergic innervation into the OB		→	n.a.	→		→	n.a.	→	
Motor symptomes	Open field (motor aspect)		→	3.8	→	14.4	→	4.3	→	15.3
Non-motor symptomes	Anxiety	Open filed test	↓	3.8	→	14.4	→	4.3	→	15.3
	Sensorimotor gating	ASR	→	4	↑^{sex}	14.7	→	4.5	→	15.5
		PPI	→		→		→		→	
	Gait	front paws	↓^{sex}	4.5	↓^{sex}	15.3	→	5.8	→	16
		hind paws	→		→		↓		↓^{sex}	
	Olfactory function	discrimination	→	6.5	→	16.1	→	6.9	→	17.7
		sensitivity	→		→		→		→	

Figure 5.20 Summary of Pink1 x Pet and Pink1 x DAT phenotypes. Pink1 x Pet and Pink1 x DAT mice were analysed with a young and mid-aged age in PD-related neuropathology, motor and non-motor symptoms. Both mouse lines showed slight non-motor symptoms related phenotypes in young and mid-aged age. n.a = not applicable, sex = only significant in one sex, → not changed, ↑ up, ↓ down, DA = dopamine, SNC = substantia nigra pars compacta, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, ASR = acoustic startle reflex, PPI = prepulse inhibition.

Chapter 6

Discussion & Outlook

Based on a previous study of Glasl and colleagues, a murine Pink1 deficiency did not lead to neuronal degeneration in the substantia nigra pars compacta (SNC) or overt motor-phenotypes. Nevertheless, selected age-dependent non-motor phenotypes in olfaction and gait, including alterations in the serotonergic system, were observed (Glasl *et al.*, 2012). This raises the question concerning the integrity of different neurotransmitter systems in Parkinson's disease (PD)-related phenotypes in young and aged animals. In addition, an *in vitro* acute knockdown (KD) of *Pink1* caused a mitochondrial phenotype, which was compensated over time. Therefore, another question focussed on the potential compensatory mechanism consequent to Pink1 deficiency (Glasl *et al.*, 2012).

In order to study:
(1) The precise contribution of the dopaminergic or serotonergic neurotransmitter systems in respect to the PD-related symptoms,
(2) The potential compensatory mechanism consequent to Pink1 deficiency, hindering the outbreak of PD and
(3) The impact of age on the outbreak of PD-related symptoms,
Two conditional *Pink1* knockout *(*KO) mouse lines were generated: the first is a mouse line with a Pink1 deficiency in dopaminergic neurons induced during early adulthood and the second line where Pink1 was constitutively eliminated in the serotonergic neurons. Both mouse lines were systemically analysed at both young adult and middle age. Animals underwent a thorough behavioural analysis with respect to PD-related motor and non-motor symptoms, as well as a neuroanatomical and biochemical analysis of the brain. In summary, neither of the mouse lines revealed PD-related neurodegeneration and neurotransmitter anomalies. However, there were some PD-related gait phenotypes. Furthermore, other behavioural testing did not show indications of strong PD-related phenotypes.

6.1 Serotonergic and dopaminergic system

The selective KO of *Pink1,* neither in the constitutive serotonergic KO nor in the inducible dopaminergic KO, led to a degeneration of dopaminergic neurons in the SNC, independent of young or middle age.

These results suggest that Pink1 deficiency in the serotonergic system does not influence dopaminergic neurons in the substantia nigra with respect to survival. This notion was conceivable taking into account that serotonergic neurons innervate the dopaminergic neurons of the substantia nigra and that serotonin might act as a neuroprotective agent (Chilmonczyk *et al.*, 2017). In addition, the number of serotonergic neurons in the raphe nuclei themselves did not succumb to cell death in the analysed mouse models (data not shown, personal communication). Furthermore, the content of dopamine and its metabolites in the analysed brain regions: olfactory bulb (OB), ventral midbrain (VM) and striatum (STR), exhibited no alterations in either of the analysed mouse lines, irrespective of age. This supports the neuropathological finding of no gross alterations in both systems in both mouse lines. However, we cannot exclude that a Pink1 deficiency has an impact on neurotransmitter release as it has been reported by several laboratories (Kitada *et al.*, 2007, Moisoi *et al.*, 2014, Gispert *et al.*, 2009, Akundi *et al.*, 2011). In order to determine such a release problem, a micro dialysis experiment would be of interest.

Considering mouse models with a constitutive KO of Parkin, Pink1, DJ-1 and even a triple KO of these three genes, none of them established degeneration of dopaminergic neurons. Hence analysis of conditional acute KO mouse models of PD are necessary in order to target the single pathways and to circumvent potential compensatory mechanisms (Kitada *et al.*, 2009, Glasl *et al.*, 2012, Perez and Palmiter, 2005, Goldberg *et al.*, 2005). Nevertheless, neither the full constitutive KO of *Pink1*, nor the inducible KO of *Pink1* in dopaminergic neurons, nor the KO of *Pink1* in serotonergic neurons induces overt neurodegeneration of dopaminergic or serotonergic neurons. Thus, at least for Pink1 deficiency there are no compensatory mechanisms established during development, which might have been overcome by the inducible KO, in contrast to the conditional *Parkin* KO. This is of specific interest since Pink1 deficiency was induced in young adulthood in the dopaminergic neurons, similar to the acute deletion of *Parkin* via lentiviral injection in mice (Shin *et al.*, 2011, Yang *et al.*, 2006). However, the absence of neuronal degeneration in the conditional Pink1 x DAT mouse line is in sharp contrast to the published inducible conditional *Parkin* KO mouse line; the acute

KO of *Parkin* indeed leads to dopaminergic neurodegeneration (Shin *et al.*, 2011). These results are surprising because Pink1 and Parkin display *in vitro* interaction, suggesting that Pink1 acts upstream of Parkin (Kondapalli *et al.*, 2012, Okatsu *et al.*, 2012, Kane *et al.*, 2014). Likewise, studies on acute *Pink1* KD flies demonstrated mitochondrial alteration and neuronal degeneration, which was suppressed by *Parkin* overexpression (Clark *et al.*, 2006, Yang *et al.*, 2006). This would entail the interacting Pink1 and Parkin in *vivo* playing a role in the development of PD-related phenotypes and pathology. However, since an acute KD of murine *Pink1* in dopaminergic neurons did not lead to neuronal degeneration, Pink1 and Parkin interaction in dopaminergic neurons might not have an impact on the development of PD-related symptoms. It is also possible that Pink1 *in vivo* in dopaminergic neurons of mice acts independently of Parkin, potentially through an additional pathway.

The absence of neurodegeneration in all these models, specifically in the *Pink1* full KO, is in sharp contrast to the human situation. Human PD patients suffering from inherited *Pink1* mutation are prone a cell loss in SNC (Puschmann, 2013). A possible explanation for the absence of neurodegeneration in Pink1-deficient mice compared to humans is the amount of neuromelanin (NM) in the SNC. NM is distributed in dopaminergic neurons and NM containing neurons of the SNC are highly vulnerable to degeneration in PD (Depboylu *et al.*, 2007). In human brains, NM is detectable whereas the presence of NM in rodent brain is under debate, which in turn indicates a higher amount of NM in the human brain (Fedorow *et al.*, 2005). Nonetheless, since dopaminergic neurons also die in rats and flies with Pink1 deficiency, there might be further factors and pathways eliciting species-specific dopaminergic neurodegeneration (Park *et al.*, 2006, Yang *et al.*, 2006, Dave *et al.*, 2014, Villeneuve *et al.*, 2016).

Aging per se is the greatest risk factor for the development of PD, since the prevalence of developing PD increases more than 400 times from 50 to 80 years of age (Collier *et al.*, 2011, Rodriguez *et al.*, 2015). Therefore, the aim was to understand the influence of age as a covariate consequent to Pink1 deficiency in selected neurotransmitter systems, since PD-related phenotypes of Pink1 deficient mice were observed in aged animals (Glasl *et al.*, 2012). Considering the number of dopaminergic neurons, a Pink1 deficiency in the serotonergic or dopaminergic system did not alter compared to the control mice. However, dopaminergic neurons do also die with increasing age in healthy persons, it is possible that the quantity of cell loss is the main difference between PD and normal aging (Rodriguez *et al.*, 2015). Independent of acute or

constitutive KO of *Pink1*, no aging effect was observed in the longitudinal study. Since the analysed mid-aged mice did not reach the age-related maximum peak of their lifetime, potential cell loss differences might have occurred at a later time point, much later than the normal life span of the mice. On the other hand, PD patients carrying a *Pink1* mutation develop an early-onset PD at an estimated age of younger than 40 years. This is much earlier than the mean age of onset of PD with about 60 years (Lees *et al.*, 2009). However, it is under debate whether potential compensatory mechanisms protect the constitutive dopaminergic neurons that die with increasing age. Overall, aging plays an important role in PD and clarifying the biological basis of aging might contribute to understanding the underlying mechanism of PD (Rodriguez *et al.*, 2015). It will be important to determine if, at a molecular level, ageing in mice can be compared to ageing in humans. This is necessary to draw definitive conclusions concerning the underlying molecular mechanisms that account for the absence of neurodegeneration in mice. If it reflects a true species-specific effect, studying mice would become even more important to be able to learn about potential compensatory mechanisms and even about neuroprotective strategies.

In summary, a KO of *Pink1* in dopaminergic and serotonergic neurons does not trigger dopaminergic neuronal degeneration nor influences the content of dopamine, serotonin and its metabolites. Since the KO of *Pink1* in dopaminergic neurons in this study was done in an acute manner at an age of 10 weeks, potential compensatory mechanisms during development could have been overcome. Thus, Pink1 in dopaminergic neurons is not involved in compensation of neuronal degeneration. In addition, Pink1 was constitutively eliminated in serotonergic neurons. The published phenotype of altered serotonergic innervation of the OB in Pink1_del2/3 mice was not replicated by the constitutive *Pink1* KO in serotonergic neurons. Hence, the serotonergic system – with Pink1 deficiency – did not contribute to the changes in serotonergic innervation in the OB. It can thus be concluded that, Pink1 is not acting in a cell autonomous manner.

6.2 Locomotor and explorative function

Gispert and colleagues described locomotor impairments in the open field (OF) testing by a reduction of distance travelled, rearing frequency and movement time in aged *Pink1* KO mice (Gispert *et al.*, 2009). Even though these mice did not display neuronal degeneration, the striatal dopamine level was altered. Pink1 deficiencies in dopaminergic and serotonergic neurons did not resemble these phenotypes. The

absence of a locomotor phenotype was likely since there was no alteration in striatal dopamine level nor were dopaminergic neurons degenerated. Indeed, first motor symptoms in PD patients were described after more than 50% of dopaminergic neurons in the SNC were lost, therefore the absence of a locomotor phenotype with Pink1 deficiency in serotonergic or dopaminergic neurons are in accordance with the neuroanatomical and biochemical results (Schapira *et al.*, 2017, Grosch *et al.*, 2016).

Interestingly, young mutant Pink1 x Pet mice rested significantly longer in the centre compared to both control groups. This is a first indication of an anxiety-related phenotype. Nevertheless, it needs to be considered that a clear anxiolytic phenotype of longer resting time in the centre might also correlate with other parameters like a longer overall resting time [s] and total distance travelled in the centre [cm] or centre average speed [cm/s]. Since these parameters were not affected in the young Pink1 x Pet mice, a clear anxiety-related behaviour is still under debate and would need to be researched with additional behavioural tests, like forced swim test or light dark box test. Still, it is known that anxiety-related behaviour is influenced by the serotonergic system (Baldwin and Rudge, 1995). Hence, Pink1 in serotonergic neurons might play a specific role in controlling anxiety-related behaviour. However, it has to be mentioned that PD patients suffer from an increased anxiety, which is in contrast to the observed anxiolytic behaviour in these mice, since anxious mice are more likely to avoid open unknown places (Jankovic, 2008, Seibenhener and Wooten, 2015). The content of serotonin did not fluctuate in the analysed brain regions: OB, VM and STR. Nevertheless, the amygdala is a key player in the development of anxiety and is prominently modulated by serotonin (Bocchio *et al.*, 2016). Therefore, further analysis on the serotonin content in the amygdala would be of interest in the sense that a specific serotonergic deficit in these brain regions might have been induced by the Pink1 deficiency.

In summary, a Pink1 deficiency in selected neurotransmitter systems did not lead to overt locomotor and explorative impairments possibly due to an insufficient induction of neuronal cell death in both neurotransmitter systems, hence no compensatory mechanism could be detected.

6.3 Acoustic startle reflex and prepulse inhibition in PD

The mid-aged male mutant Pink1 x Pet mice startled significantly more compared to both control groups on stimulation by the loud sound pressure of 120 dB. Nevertheless,

the relevance of this finding has to be ascertained. These results are in agreement with studies on a PD patient with a *Pink1* mutation, who exhibited hyperreflexia symptoms (Zhang *et al.*, 2005). It is known that serotonin is involved in reflex modulation (Schmidt and Jordan, 2000). However, further studies would be necessary, in order to understand the serotonergic influence in Pink1 deficient mice on possible reflex-related parameters, such as nerve conductance velocity testing or electrophysiological analysis to study the function of reflex arcs.

On the other hand, this phenotype could also be due to mutant mice exhibiting less hearing loss compared to both control groups upon ageing. Indeed, serotonin plays an important role in the neuronal circuit in hearing processing. Human studies have shown that citalopram, a serotonin reuptake inhibitor, has a positive impact on the acoustic processing in older people (Cruz *et al.*, 2004). In addition, it is known that a decreased serotonergic receptor expression correlates with the age-dependent hearing loss (Tadros *et al.*, 2007). Hence, a Pink1 deficiency possibly influences the gene expression of these serotonergic receptors and may explain why mutant mice undergo less hearing loss. However, post mortem studies on PD patients revealed an increased number of serotonergic receptors (Ballanger *et al.*, 2010), which is contradictory to the hypothesis above. Thus, further analysis of serotonergic receptors in Pink1 x Pet mice would be of interest, in order to discern a potential correlation of serotonergic receptors to hearing loss. Nevertheless, improved hearing capability consequent to a Pink1 deficiency is contradictory to studies on PD patients, who develop age-dependent peripheral hearing impairments (Vitale *et al.*, 2012).

6.4 Gait in PD

As a rough overview, Pink1 x Pet (female) mutant mice exhibited significant differences compared to the control groups in the parameter Max contact at [%] of the (left) front paws and temporal relation of the front paws, described by Phase dispersion [%]. The mutant Pink1 x DAT mice showed significant differences in parameters concerned with speed and the position of the footprint *i.e.* the base of support.

Gait alterations were already described in PD patients with *Pink1* mutations (Siuda *et al.*, 2014). Interestingly, the Pink1_del2/3 mice, which were tested in the study of Glasl and colleagues, exhibited similar phenotypes (Glasl *et al.*, 2012). Male Pink1 deficient mice tended to exhibit a higher Max Contact [%] compared to the control mice. Referring to the present study, female mutant Pink1 x Pet mice showed a significant higher Max Contact [%] compared to the control. It needs to be considered that the

hind paws of male Pink1 deficient mice were affected in contrast to the affected female (left) front paws of mutant Pink1 x Pet mice. In addition, male Pink1 deficient mice showed impairments in phase dispersion of diagonal paws. The parameter phase dispersion was also affected in the female mutant Pink1 x Pet mice. However, female front paws were affected.

Pink1 x DAT male mutant mice showed a tendency and significant difference compared to both control groups in a lower base of support. This parameter was also significantly reduced in the male Pink1_del2/3 mice published by Glasl and colleagues, showing a dopamine dependent gait phenotype (Glasl *et al.*, 2012). In support of that, treatment of PD patients with L-3.4-Dihydroxyphenylalanine (L-DOPA) improved the kinematic parameter swing velocity, which was significantly reduced in mutant Pink1 x DAT mice (Blin *et al.*, 1991). In another study of PD patients, dopamine depletion was shown to impair gait automaticity, showing a gait dependency on dopamine (Gilat *et al.*, 2017).

Considering the procedure of CatWalk testing, mice receive no prior training, which might result in higher variations within the different runs and a lowered power of significance. Since several parameters were analysed in the CatWalk testing, the possibility of false positive results can increase. Therefore, in order to interpret data from CatWalk analysis in a robust manner, new untested cohorts exhibiting a Pink1 deficiency in the selected neurotransmitter systems would need to be tested on a CatWalk system to confirm the observed PD-related gait phenotypes. It needs to be considered that Pink1_del2/3 mice were tested on another CatWalk system compared to mice in the present study. This could influence the comparison of the different mouse lines because results of both CatWalk versions differ (Zimprich *et al.*, 2017). In order to understand the present gait phenotype in Pink1 deficient mice, further morphological and functional studies on the neuronal network with respect to gait would be of interest.

In sum, a Pink1 deficiency in serotonergic and dopaminergic systems plays a role in gait, which was already described in the *Pink1* KO mice in the study of Glasl and colleagues (Glasl *et al.*, 2012). Interestingly, parameters that were affected in the Pink1_del2/3 mice were described in both analysed mouse lines, indicating that the serotonergic and dopaminergic system are involved in PD-related gait impairments consequent to Pink1 deficiency.

It is important to note that in contrast to the altered gait parameters in the two lines studied in this thesis, the full KO of *Pink1* exhibited a more robust pattern of gait alterations. Thus, it is highly likely that beyond the dopaminergic and serotonergic

system also other systems are involved. In this respect it has to be mentioned that Pink1 is most strongly expressed in muscles (mammalian walking recruits up to 80 individual muscles, which generates a high complexity of unique burst pattern) (Sharples *et al.*, 2014) as well as in interneurons and motoneurons in the spinal cord (d'Amora *et al.*, 2011, Morimoto *et al.*, 2010). Hence, a Pink1 deficiency might play an important role in gait performance, since muscles per se could be impaired.

Dopamine and serotonin play an important role in the neuronal modulation of the spinal cord in order to produce locomotor patterns, which are essential for responding to environmental conditions (Sharples *et al.*, 2014, Harris-Warrick and Cohen, 1985). Dopaminergic and serotonergic neurons were found in the spinal cord, in addition various serotonergic populations of the brainstem innervate the spinal cord (Hou *et al.*, 2016, Ghosh and Pearse, 2014). Dopamine influences the central pattern generator (CPG) in a dose-dependent opposing manner. On the one hand, high doses of dopamine recruits the D1 receptors and mediates an excitatory action, whereas low doses of dopamine recruits D2 receptors and mediates an inhibitory action (Clemens *et al.*, 2012). In addition, dopamine modulates the locomotor rhythm by influencing multiple molecular targets in motor neurons and interneurons (Sharples, 2017). Serotonin acts on the motor neurons and interneurons by binding to different types of serotonergic receptors in the spinal cord in order to control the CPG activation, motor neuron output (via 5-HT_2 receptors) and locomotion of the CPG (via 5-HT_7 receptors) (Slawinska *et al.*, 2014). Likewise, serotonin acts in an excitatory and inhibitory action on the motor neurons (Wang and Dun, 1990). Impairments of the serotonergic system during the perinatal period causes alterations in locomotion and motor neuron innervation (Pearlstein *et al.*, 2011). Since the Pink1 deficiency in serotonergic neurons was constitutive, a potential alteration in serotonin release could be a reason for the observed gait alteration. Even though serotonin and dopamine levels in the brain were not altered in the OB, VM and STR, the peripheral neurotransmitter content and release were not quantified. It is possible that minor neurotransmitter changes might impose gross locomotor alterations, since for example dopamine acts in a dose dependent opposing manner on the CPG (Clemens *et al.*, 2012). Thus, further analysis of the neurotransmitter content in the spinal cord would be of interest. Interestingly, non-motor gait alterations were observed in young and mid-aged mice upon a Pink1 deficiency, indicating an age independent development of PD-related phenotypes.

6.5 Olfaction in Parkinson's disease

As described in the analysis of Pink1_del2/3 mice in the study of Glasl and colleagues, it was shown that a murine Pink1 deficiency caused impairments in discrimination of binary mixtures and olfactory sensitivity (Glasl *et al.*, 2012). Nevertheless, a selective KO of *Pink1* in dopaminergic nor serotonergic neurons did not replicate the previously published data. This meant that there was no significant influence on the olfactory capability with Pink1 deficiency in the specific neurotransmitter systems. Furthermore, the innervation of the olfactory bulb by serotonergic nerve fibres was not changed. The latter is in contrast to the Pink1_del2/3 mice, which indicates that the decreased serotonergic innervation is not due to a deficiency of Pink1 in the serotonergic neurons (Glasl *et al.*, 2012). It might however be influenced by an altered environment in the OB due to Pink1 deficiency, since Pink1 is highly expressed in the OB (Taymans *et al.*, 2006). In any case, this point would need further investigation.

It would be conceivable that the described olfactory impairments in Pink1_del2/3 mice are caused possibly by the impaired serotonergic innervation in the OB. This is in agreement with previous analysis of serotonergic fibres, which influence for example the dopaminergic and γ-Aminobutyric acid (GABA)ergic periglomerular interneurons in the OB. They in turn regulate the olfactory sensory neurons and the subsequent olfactory processing (Dugue and Mainen, 2009). On the other hand, dopamine might not play a key role in the establishment of olfactory problems and hyposmia in PD patients. This is because the degree of hyposmia is independent of the stage of dopaminergic degeneration and the amount of L-DOPA treatment (Doty, 2012).

Since no strong olfactory phenotype was observed in the mice of the present study, the underlying mechanism of the previously described phenotype in Pink1_del2/3 mice is still under debate. The Pink1_del2/3 mice were tested at an older age (27 months) compared to the mice of the present study. Olfactory dysfunction increases with age, with the result that a possible effect in the tested mice of the present study was missed due to the younger age (Doty and Kamath, 2014). Zhou and colleagues figured out that PD patients with an older age of onset were characterized by more olfactory symptoms compared to early-onset PD patients (Zhou *et al.*, 2013). Possibly mutant Pink1 x Pet and Pink1 x DAT mice would also exhibit (if at all) only minor olfactory impairments if tested at an age older than 20 months, supported by the mild olfactory impairments in *Pink1* PD patients and Pink1_del2/3 mice (Zhou *et al.*, 2013, Doty, 2012, Glasl *et al.*, 2012). In order to figure out possible mild olfactory impairments in mutant Pink1 x Pet and Pink1 x DAT mice, a higher number of tested animals would

be of benefit. Olfactory impairments were already described in other PD mouse models, like ATP13a2 KO mouse model by another olfactory test, the “Burried Pellet Test”, which gives information about the overall olfactory ability but not about fine discrimination and olfactory sensitivity (Lehmkuhl *et al.*, 2014). In sum, olfactory testing of PD mouse models is valuable to yield more information about potential non-motor symptoms. Nonetheless, it needs to be considered that it is challenging to discover mild olfactory impairments. Apart from the possible structural and functional impairment of the OB, Pink1 deficient mice could also exhibit impairments in higher brain regions for olfactory processing like, for example, the anterior olfactory nucleus and piriform and periamygdaloid cortex (Doty, 2012) for which Pink1 deficiency in dopaminergic and serotonergic neurons is – based on our results – not decisive.

6.7 Concluding remarks

The questions of the study were to discern the potential contribution of the serotonergic and dopaminergic system to the PD-related phenotypes consequent to Pink1 deficiency and to reveal potential compensatory mechanisms, including the impact of age. For this purpose, two conditional mouse lines were analysed, exhibiting a selective KO of *Pink1* in serotonergic or dopaminergic neurons. Neither of the mouse lines exhibited clear PD-related pathologies nor motor phenotypes. However, some slight alterations in the non-motor phenotype “gait” were observed in both mouse lines, independent of age. Overall the serotonergic and dopaminergic system are involved in the PD-related gait phenotypes with Pink1 deficiency. However, no gross compensatory mechanism could be uncovered including the absence of a PD-related phenotype, which occurred in an age-dependent manner. Nevertheless, Pink1 x Pet and Pink1 x DAT mouse lines are useful mouse models of the prodromal phase of PD in order to study non-motor symptoms concerning gait. Since PD is a multifactorial disease, potential PD-related phenotypes in the Pink1 x Pet and Pink1 x DAT mice were hidden due to the lack of a stressor (Sulzer, 2007). In order to mimic the multifactorial nature of the disease, analysis of these mice under stressful conditions would be of interest. Since non-motor symptoms in PD patients are likely to occur much earlier than the motor symptoms, further studies in the mouse models exhibiting prodromal symptoms of PD might be instrumental for developing new preventive measures, disease halting drugs as well as new diagnostic tools for early diagnosis of PD.

Appendix

Table A1: Overview of analysed young male Pink1 x Pet mice of the cohort 1

male Pink1 x Pet, cohort 1																
						young										
						OF		ASR/PPI		Catwalk		Olfaction				
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age
30132737	160	30319982	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	16.12.2014	4.7				
30132739	162	30319983	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	16.12.2014	4.7				
30132758	177	30319984	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	20.11.2014	3.9	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.2	25.02.2015	7.1
30132976	194	30319985	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	20.11.2014	3.9	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.2	25.02.2015	7.1
30133497	218	30319986	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	20.11.2014	3.7	01.12.2014	4.1	15.12.2014	4.6	28.01.2015	6.0	25.02.2015	7.0
30133511	232	30319987	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	24.11.2014	3.9	01.12.2014	4.1	12.12.2014	4.5	28.01.2015	6.0	25.02.2015	7.0
30133538	268	30319988	m	Cre:Cre+; Pink1:wt/wt	30.07.2014	20.11.2014	3.8	01.12.2014	4.1	15.12.2014	4.6	28.01.2015	6.1	25.02.2015	7.0
30133543	273	30319989	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	24.11.2014	3.9	01.12.2014	4.1	16.12.2014	4.6				
30133545	275	30319990	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	24.11.2014	3.9	01.12.2014	4.1	16.12.2014	4.6				
30133553	281	30319991	m	Cre:Cre+; Pink1:wt/wt	01.08.2014	20.11.2014	3.7	01.12.2014	4.1	12.12.2014	4.4	28.01.2015	6.0	25.02.2015	6.9
30133560	288	30319992	m	Cre:Cre+; Pink1:wt/wt	01.08.2014	20.11.2014	3.7	01.12.2014	4.1	12.12.2014	4.4	28.01.2015	6.0	25.02.2015	6.9
30135140	396	30319994	m	Cre:Cre+; Pink1:wt/wt	23.08.2014	20.11.2014	3.0	01.12.2014	3.3	16.12.2014	3.8				
30133601	319	30319995	m	Cre:Cre+; Pink1:wt/wt	02.08.2014	20.11.2014	3.7	01.12.2014	4.0	15.12.2014	4.5	28.01.2015	6.0	25.02.2015	6.9
30132719	152	30319966	m	Cre:Cre+; Pink1:flox/flox	28.07.2014	20.11.2014	3.8	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.1	25.02.2015	7.1
30132752	171	30319967	m	Cre:Cre+; Pink1:flox/flox	28.07.2014	20.11.2014	3.8	01.12.2014	4.2	12.12.2014	4.6				
30132756	175	30319968	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	20.11.2014	3.9	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.2	25.02.2015	7.1
30132759	178	30319969	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	20.11.2014	3.9	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.2	25.02.2015	7.1
30132975	193	30319970	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	20.11.2014	3.9	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.2	25.02.2015	7.1
30132978	196	30319971	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	16.12.2014	4.7				
30133014	211	30319972	m	Cre:Cre+; Pink1:flox/flox	29.07.2014	20.11.2014	3.8	01.12.2014	4.2	12.12.2014	4.5				
30133523	253	30319973	m	Cre:Cre+; Pink1:flox/flox	31.07.2014	24.11.2014	3.9	01.12.2014	4.1	15.12.2014	4.6				
30133533	263	30319974	m	Cre:Cre+; Pink1:flox/flox	30.07.2014	24.11.2014	3.9	01.12.2014	4.1	15.12.2014	4.6	28.01.2015	6.1	25.02.2015	7.0
30133579	308	30319975	m	Cre:Cre+; Pink1:flox/flox	01.08.2014	24.11.2014	3.8	01.12.2014	4.1	15.12.2014	4.5	28.01.2015	6.0	25.02.2015	6.9
30133761	349	30319976	m	Cre:Cre+; Pink1:flox/flox	03.08.2014	24.11.2014	3.8	01.12.2014	4.0	16.12.2014	4.5	28.01.2015	5.9	25.02.2015	6.9
30133765	353	30319977	m	Cre:Cre+; Pink1:flox/flox	03.08.2014	24.11.2014	3.8	01.12.2014	4.0	16.12.2014	4.5	28.01.2015	5.9	25.02.2015	6.9
	439	30146250	m	Cre:Cre+; Pink1:flox/flox	29.01.2015										
	589	30147537	m	Cre:Cre+; Pink1:flox/flox	14.02.2015										
30132721	154	30319956	m	Cre:Cre-; Pink1:flox/flox	28.07.2014	20.11.2014	3.8	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.1	25.02.2015	7.1
30132722	155	30319957	m	Cre:Cre-; Pink1:flox/flox	28.07.2014	20.11.2014	3.8	01.12.2014	4.2	12.12.2014	4.6	28.01.2015	6.1	25.02.2015	7.1
30133505	226	30319958	m	Cre:Cre-; Pink1:flox/flox	31.07.2014	24.11.2014	3.9	01.12.2014	4.1	12.12.2014	4.5	28.01.2015	6.0	25.02.2015	7.0
30133568	296	30319959	m	Cre:Cre-; Pink1:flox/flox	01.08.2014	20.11.2014	3.7	01.12.2014	4.1	12.12.2014	4.4	28.01.2015	6.0	25.02.2015	6.9
30133584	313	30319960	m	Cre:Cre-; Pink1:flox/flox	01.08.2014	24.11.2014	3.8	01.12.2014	4.1	15.12.2014	4.5	28.01.2015	6.0	25.02.2015	6.9
30133610	328	30319961	m	Cre:Cre-; Pink1:flox/flox	02.08.2014	20.11.2014	3.7	01.12.2014	4.0	15.12.2014	4.5	28.01.2015	6.0	25.02.2015	6.9
30134063	368	30319962	m	Cre:Cre-; Pink1:flox/flox	07.08.2014	24.11.2014	3.6	01.12.2014	3.9	16.12.2014	4.4				
30135122	389	30319963	m	Cre:Cre-; Pink1:flox/flox	24.08.2014	20.11.2014	2.9	01.12.2014	3.3	16.12.2014	3.8	28.01.2015	5.2	25.02.2015	6.2
30135123	390	30319964	m	Cre:Cre-; Pink1:flox/flox	24.08.2014	20.11.2014	2.9	01.12.2014	3.3	16.12.2014	3.8	28.01.2015	5.2	25.02.2015	6.2
	508	30146318	m	Cre:Cre-; Pink1:flox/flox	31.01.2015										
	525	30146335	m	Cre:Cre-; Pink1:flox/flox	01.02.2015										

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. Some movies of CatWalk testing (red colour) could not be analysed and were not considered for the analysis. Some animals, tested in olfaction testing, could not be considered for the analysis (red colour). OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition. The age is expressed in months.

Table A2: Overview of analysed mid-aged male Pink1 x Pet mice of the cohort 1

male Pink1 x Pet, cohort 1																									
						mid-aged																			
						OF		ASR/PPI		Catwalk		Olfaction				prep		TH	HPLC			5-HT	
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age	date	age	date est.	date OB	date STR	date VM	date	
30132737	160	30319982	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	06.10.2015	14.5	13.10.2015	20.0	02.11.2015	15.4					17.03.2016	19.97	01.06.2016	16.06.2016	04.07.2016	30.06.2016		
30132739	162	30319983	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	06.10.2015	14.5	13.10.2015	14.8	02.11.2015	15.4					17.03.2016	19.97		16.06.2016	05.07.2016	01.07.2016		
30132758	177	30319984	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	06.10.2015	14.5	13.10.2015	14.8	02.11.2015	15.4	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.97	01.06.2016	17.06.2016	05.07.2016	01.07.2016		
30132976	194	30319985	m	Cre:Cre+; Pink1:wt/wt	27.07.2014	06.10.2015	14.5	12.10.2015	14.7	03.11.2015	15.5	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.97		20.06.2016	06.07.2016	30.06.2016		
30133497	218	30319986	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	06.10.2015	14.4	13.10.2015	14.6	03.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.5	17.03.2016	19.83	01.06.2016	20.06.2016	05.07.2016	04.07.2016		
30133511	232	30319987	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	06.10.2015	14.4	12.10.2015	14.6	03.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.5								
30133538	268	30319988	m	Cre:Cre+; Pink1:wt/wt	30.07.2014	06.10.2015	14.4	13.10.2015	14.7	03.11.2015	15.4	17.11.2015	15.8	07.12.2015	16.5								
30133543	273	30319989	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	06.10.2015	14.4	13.10.2015	14.6	03.11.2015	15.3					17.03.2016	19.83	01.06.2016	20.06.2016	04.07.2016	04.07.2016		
30133545	275	30319990	m	Cre:Cre+; Pink1:wt/wt	31.07.2014	06.10.2015	14.4	13.10.2015	14.6	03.11.2015	15.3					17.03.2016	19.83	01.06.2016	27.06.2016	05.07.2017	01.07.2016		
30133553	281	30319991	m	Cre:Cre+; Pink1:wt/wt	01.08.2014			12.10.2015	14.6	03.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.4	17.03.2016	19.80	01.06.2016					
30133560	288	30319992	m	Cre:Cre+; Pink1:wt/wt	01.08.2014	06.10.2015	14.4	12.10.2015	14.6	04.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.4	17.03.2016	19.80	01.06.2016	27.06.2016	01.08.2016	30.06.2016		
30135140	396	30319994	m	Cre:Cre+; Pink1:wt/wt	23.08.2014																		
30133601	319	30319995	m	Cre:Cre+; Pink1:wt/wt	02.08.2014	06.10.2015	14.3	12.10.2015	14.5	04.11.2015	15.3	17.11.2015	15.7	07.12.2015	16.4								
30132719	152	30319966	m	Cre:Cre+; Pink1:flox/flox	28.07.2014	06.10.2015	14.5	12.10.2015	14.7	02.11.2015	15.4	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.93	01.06.2016					
30132752	171	30319967	m	Cre:Cre+; Pink1:flox/flox	28.07.2014																		
30132756	175	30319968	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	06.10.2015	14.5	13.10.2015	14.8	02.11.2015	15.4	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.97		16.06.2016	05.07.2016	30.06.2016		
30132759	178	30319969	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	06.10.2015	14.5	13.10.2015	14.8	02.11.2015	15.4	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.97	01.06.2016	16.06.2016	06.08.2016	01.07.2016		
30132975	193	30319970	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	06.10.2015	14.5	13.10.2015	14.8	02.11.2015	15.4	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.97	01.06.2016	16.06.2016	05.07.2016	30.06.2016		
30132978	196	30319971	m	Cre:Cre+; Pink1:flox/flox	27.07.2014	06.10.2015	14.5	13.10.2015	14.8	03.11.2015	15.5					17.03.2016	19.97	01.06.2016	20.06.2016	06.07.2016	01.07.2016		
30133014	211	30319972	m	Cre:Cre+; Pink1:flox/flox	29.07.2014	06.10.2015	14.5	13.10.2015	14.7	03.11.2015	15.4					17.03.2016	19.90		20.06.2016	05.07.2016	04.07.2016		
30133523	253	30319973	m	Cre:Cre+; Pink1:flox/flox	31.07.2014	06.10.2015	14.4	13.10.2015	14.6	03.11.2015	15.3					17.03.2016	19.83	01.06.2016	20.06.2016	06.07.2016	01.07.2016		
30133533	263	30319974	m	Cre:Cre+; Pink1:flox/flox	30.07.2014	06.10.2015	14.4	13.10.2015	14.7	03.11.2015	15.4	17.11.2015	15.8	07.12.2015	16.5	17.03.2016	19.87	01.06.2016	27.06.2016	05.07.2016	01.07.2016		
30133579	308	30319975	m	Cre:Cre+; Pink1:flox/flox	01.08.2014	06.10.2015	14.4	12.10.2015	14.6	03.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.4								
30133761	349	30319976	m	Cre:Cre+; Pink1:flox/flox	03.08.2014	06.10.2015	14.3	13.10.2015	14.5	03.11.2015	15.2	17.11.2015	15.7	07.12.2015	16.4	17.03.2016	19.73	01.06.2016	27.06.2016	05.07.2016	01.07.2016		
30133765	353	30319977	m	Cre:Cre+; Pink1:flox/flox	03.08.2014	06.10.2015	14.3	14.10.2015	14.6	04.11.2015	15.3	17.11.2015	15.7	07.12.2015	16.4								
	439	30146250	m	Cre:Cre+; Pink1:flox/flox	29.01.2015											08.09.2016	19.60					06.10.2016	
	589	30147537	m	Cre:Cre+; Pink1:flox/flox	14.02.2015											08.09.2016	19.07					06.10.2016	
30132721	154	30319956	m	Cre:Cre-; Pink1:flox/flox	28.07.2014	06.10.2015	14.5	12.10.2015	14.7	02.11.2015	15.4	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.93	01.06.2016	17.06.2016	04.07.2016	04.07.2016		
30132722	155	30319957	m	Cre:Cre-; Pink1:flox/flox	28.07.2014	06.10.2015	14.5	12.10.2015	14.7	02.11.2015	15.4	17.11.2015	15.9	07.12.2015	16.6	17.03.2016	19.93	01.06.2016	17.06.2016	05.07.2016	30.06.2016		
30133505	226	30319958	m	Cre:Cre-; Pink1:flox/flox	31.07.2014	06.10.2015	14.4	12.10.2015	14.6	02.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.5	17.03.2016	19.83	01.06.2016	16.06.2016	06.07.2016	01.07.2016		
30133568	296	30319959	m	Cre:Cre-; Pink1:flox/flox	01.08.2014	06.10.2015	14.4	12.10.2015	14.6	02.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.4	17.03.2016	19.80	01.06.2016	20.06.2016	05.07.2016	30.06.2016		
30133584	313	30319960	m	Cre:Cre-; Pink1:flox/flox	01.08.2014	06.10.2015	14.4	12.10.2015	14.6	03.11.2015	15.3	17.11.2015	15.8	07.12.2015	16.4	17.03.2016	19.80	01.06.2016	22.06.2016	04.07.2016	01.07.2016		
30133610	328	30319961	m	Cre:Cre-; Pink1:flox/flox	02.08.2014	06.10.2015	14.3	12.10.2015	14.5	03.11.2015	15.3	17.11.2015	15.7	07.12.2015	16.4	17.03.2016	19.77	01.06.2016	20.06.2016	05.07.2016	04.07.2016		
30134063	368	30319962	m	Cre:Cre-; Pink1:flox/flox	07.08.2014	06.10.2015	14.2	12.10.2015	14.4	03.11.2015	15.1												
30135122	389	30319963	m	Cre:Cre-; Pink1:flox/flox	24.08.2014	06.10.2015	13.6	12.10.2015	13.8	03.11.2015	14.5	17.11.2015	15.0	07.12.2015	15.7	17.03.2016	19.03	01.06.2016	27.06.2016	05.07.2016	01.07.2016		
30135123	390	30319964	m	Cre:Cre-; Pink1:flox/flox	24.08.2014	06.10.2015	13.6	12.10.2015	13.8	03.11.2015	14.5	17.11.2015	15.0	07.12.2015	15.7	17.03.2016	19.03	01.06.2016	27.06.2016	05.07.2016	01.07.2016		
	508	30146318	m	Cre:Cre-; Pink1:flox/flox	31.01.2015											08.09.2016	19.53					06.10.2016	
	525	30146335	m	Cre:Cre-; Pink1:flox/flox	01.02.2015											08.09.2016	19.50					06.10.2016	

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. Some movies of CatWalk testing (red colour) could not be analysed and were not considered for the analysis. Some animals, tested in olfaction testing, could not be considered for the analysis (red colour). OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition, TH = immunohistochemical staining of TH^+ cells, 5-HT = immunohistochemical staining of 5-HT^+ cells, HPLC = quantification of neurotransmitter content via HPLC, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, date est. = estimated date. The age is expressed in months.

Table A3: Overview of analysed young female Pink1 x Pet mice of the cohort 1

female Pink1 x Pet, cohort 1															
						young									
						OF		ASR/PPI		Catwalk		Olfaction			
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age
30132724	157	30320025	f	Cre:Cre+; Pink1:wt/wt	28.07.2014	25.11.2014	4.0	02.12.2014	4.2	18.12.2014	4.8				
30132745	168	30320026	f	Cre:Cre+; Pink1:wt/wt	27.07.2014	24.11.2014	4.0	02.12.2014	4.3	17.12.2014	4.8				
30132769	188	30320027	f	Cre:Cre+; Pink1:wt/wt	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	17.12.2014	4.8				
30132772	191	30320028	f	Cre:Cre+; Pink1:wt/wt	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	16.12.2014	4.7				
30133503	224	30320029	f	Cre:Cre+; Pink1:wt/wt	31.07.2014	25.11.2014	3.9	02.12.2014	4.1	18.12.2014	4.7				
30133556	284	30320030	f	Cre:Cre+; Pink1:wt/wt	01.08.2014	25.11.2014	3.9	02.12.2014	4.1	17.12.2014	4.6				
30133588	317	30320031	f	Cre:Cre+; Pink1:wt/wt	01.08.2014	25.11.2014	3.9	02.12.2014	4.1	17.12.2014	4.6				
30133589	318	30320032	f	Cre:Cre+; Pink1:wt/wt	01.08.2014	25.11.2014	3.9	02.12.2014	4.1	18.12.2014	4.6				
30133611	329	30320033	f	Cre:Cre+; Pink1:wt/wt	02.08.2014	25.11.2014	3.8	02.12.2014	4.1	19.12.2014	4.6				
30133612	330	30320034	f	Cre:Cre+; Pink1:wt/wt	02.08.2014	25.11.2014	3.8	02.12.2014	4.1	19.12.2014	4.6				
30133763	351	30320035	f	Cre:Cre+; Pink1:wt/wt	03.08.2014	25.11.2014	3.8	02.12.2014	4.0	19.12.2014	4.6				
30133764	352	30320036	f	Cre:Cre+; Pink1:wt/wt	03.08.2014	25.11.2014	3.8	02.12.2014	4.0	19.12.2014	4.6				
30135108	386	30320037	f	Cre:Cre+; Pink1:wt/wt	24.08.2014	25.11.2014	3.1	02.12.2014	3.3	17.12.2014	3.8				
30135128	395	30320038	f	Cre:Cre+; Pink1:wt/wt	24.08.2014	24.11.2014	3.1	02.12.2014	3.3	18.12.2014	3.9				
30132711	151	30320010	f	Cre:Cre+; Pink1:flox/flox	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	16.12.2014	4.7				
30132743	166	30320011	f	Cre:Cre+; Pink1:flox/flox	27.07.2014	24.11.2014	4.0	02.12.2014	4.3	17.12.2014	4.8				
30132770	189	30320012	f	Cre:Cre+; Pink1:flox/flox	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	17.12.2014	4.8				
30133018	215	30320013	f	Cre:Cre+; Pink1:flox/flox	29.07.2014	25.11.2014	4.0	02.12.2014	4.2	17.12.2014	4.7				
30133502	223	30320014	f	Cre:Cre+; Pink1:flox/flox	31.07.2014	25.11.2014	3.9	02.12.2014	4.1	18.12.2014	4.7				
30133517	238	30320015	f	Cre:Cre+; Pink1:flox/flox	31.07.2014	25.11.2014	3.9	02.12.2014	4.1	18.12.2014	4.7				
30133555	283	30320016	f	Cre:Cre+; Pink1:flox/flox	01.08.2014	25.11.2014	3.9	02.12.2014	4.1	17.12.2014	4.6				
30133769	357	30320017	f	Cre:Cre+; Pink1:flox/flox	03.08.2014	24.11.2014	3.8	02.12.2014	4.0	19.12.2014	4.6				
30134065	370	30320018	f	Cre:Cre+; Pink1:flox/flox	07.08.2014	25.11.2014	3.7	02.12.2014	3.9	16.12.2014	4.4				
30135109	387	30320019	f	Cre:Cre+; Pink1:flox/flox	24.08.2014	25.11.2014	3.1	02.12.2014	3.3	17.12.2014	3.8				
30135124	391	30320020	f	Cre:Cre+; Pink1:flox/flox	24.08.2014	24.11.2014	3.1	02.12.2014	3.3	18.12.2014	3.9				
	577	30146744	f	Cre:Cre+; Pink1:flox/flox	08.02.2015										
	655	30157188	f	Cre:Cre+; Pink1:flox/flox	12.06.2015										
30132708	148	30319996	f	Cre:Cre-; Pink1:flox/flox	27.07.2014	24.11.2014	4.0	01.12.2014	4.2	16.12.2014	4.7				
30132755	174	30319997	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	24.11.2014	4.0	01.12.2014	4.2	16.12.2014	4.7				
30132768	187	30319998	f	Cre:Cre-; Pink1:flox/flox	27.07.2014	24.11.2014	4.0	02.12.2014	4.3	17.12.2014	4.8				
30132981	199	30319999	f	Cre:Cre-; Pink1:flox/flox	27.07.2014	24.11.2014	4.0	02.12.2014	4.3	17.12.2014	4.8				
30132996	203	30320000	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	25.11.2014	4.0	02.12.2014	4.2	17.12.2014	4.7				
30132997	204	30320001	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	25.11.2014	4.0	02.12.2014	4.2	17.12.2014	4.7				
30133016	213	30320002	f	Cre:Cre-; Pink1:flox/flox	29.07.2014	25.11.2014	4.0	02.12.2014	4.2	17.12.2014	4.7				
30133510	231	30320003	f	Cre:Cre-; Pink1:flox/flox	31.07.2014	25.11.2014	3.9	02.12.2014	4.1	18.12.2014	4.7				
30133527	257	30320004	f	Cre:Cre-; Pink1:flox/flox	31.07.2014	25.11.2014	3.9	02.12.2014	4.1	19.12.2014	4.7				
30133528	258	30320005	f	Cre:Cre-; Pink1:flox/flox	31.07.2014	25.11.2014	3.9	02.12.2014	4.1	19.12.2014	4.7				
30135126	393	30320006	f	Cre:Cre-; Pink1:flox/flox	24.08.2014	24.11.2014	3.1	02.12.2014	3.3	18.12.2014	3.9				
30135144	401	30320007	f	Cre:Cre-; Pink1:flox/flox	23.08.2014	25.11.2014	3.1	02.12.2014	3.4	19.12.2014	3.9				
30133000	207	30320008	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	25.11.2014	4.0	02.12.2014	4.2	18.12.2014	4.8				
30133001	208	30320009	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	25.11.2014	4.0	02.12.2014	4.2	18.12.2014	4.8				
	492	30146303	f	Cre:Cre-; Pink1:flox/flox	31.01.2015										
	576	30146743	f	Cre:Cre-; Pink1:flox/flox	07.02.2015										

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. Some movies of CatWalk testing (labelled in red colour) could not be analysed and were not considered for the analysis. OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition. The age is expressed in months.

Table A4: Overview of analysed mid-aged female Pink1 x Pet mice of the cohort 1

female Pink1 x Pet, cohort 1																								
						mid-aged																		
						OF		ASR/PPI		Catwalk		Olfaction				prep		TH	HPLC			5-HT	
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age	date	age	date est.	date OB	date STR	date VM	date	
30132724	157	30320025	f	Cre:Cre+; Pink1:wt/wt	28.07.2014	07.10.2015	14.5																
30132745	168	30320026	f	Cre:Cre+; Pink1:wt/wt	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00		16.06.2016	04.07.2016	30.06.2016		
30132769	188	30320027	f	Cre:Cre+; Pink1:wt/wt	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00	01.06.2016	16.06.2016	05.07.2016	01.07.2016		
30132772	191	30320028	f	Cre:Cre+; Pink1:wt/wt	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00						
30133503	224	30320029	f	Cre:Cre+; Pink1:wt/wt	31.07.2014	07.10.2015	14.4	14.10.2015	14.7	04.11.2015	15.4												
30133556	284	30320030	f	Cre:Cre+; Pink1:wt/wt	01.08.2014	07.10.2015	14.4	14.10.2015	14.6	04.11.2015	15.3					18.03.2016	19.83	01.06.2016	16.06.2016	01.08.2016	01.07.2016		
30133588	317	30320031	f	Cre:Cre+; Pink1:wt/wt	01.08.2014	07.10.2015	14.4	14.10.2015	14.6	05.11.2015	15.4					18.03.2016	19.83	01.06.2016					
30133589	318	30320032	f	Cre:Cre+; Pink1:wt/wt	01.08.2014	07.10.2015	14.4	14.10.2015	14.6	05.11.2015	15.4												
30133611	329	30320033	f	Cre:Cre+; Pink1:wt/wt	02.08.2014	07.10.2015	14.4	14.10.2015	14.6	05.11.2015	15.3					18.03.2016	19.80	01.06.2016	20.06.2016	04.07.2016	04.07.2016		
30133612	330	30320034	f	Cre:Cre+; Pink1:wt/wt	02.08.2014																		
30133763	351	30320035	f	Cre:Cre+; Pink1:wt/wt	03.08.2014	07.10.2015	14.3	14.10.2015	14.6	05.11.2015	15.3					18.03.2016	19.77		20.06.2016	05.07.2016	01.07.2016		
30133764	352	30320036	f	Cre:Cre+; Pink1:wt/wt	03.08.2014	07.10.2015	14.3	14.10.2015	14.6	05.11.2015	15.3					18.03.2016	19.77	01.06.2016	27.06.2016	05.07.2016	01.07.2016		
30135108	386	30320037	f	Cre:Cre+; Pink1:wt/wt	24.08.2014	07.10.2015	13.6	14.10.2015	13.9	05.11.2015	14.6					18.03.2016	19.07	01.06.2016	20.06.2016	05.07.2016	04.07.2016		
30135128	395	30320038	f	Cre:Cre+; Pink1:wt/wt	24.08.2014	07.10.2015	13.6	14.10.2015	13.9	06.11.2015	14.6					18.03.2016	19.07	01.06.2016	27.06.2016	06.07.2016	30.06.2016		
30132711	151	30320010	f	Cre:Cre+; Pink1:flox/flox	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00						
30132743	166	30320011	f	Cre:Cre+; Pink1:flox/flox	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00	01.06.2016	17.06.2016	06.07.2016	30.06.2016		
30132770	189	30320012	f	Cre:Cre+; Pink1:flox/flox	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00	01.06.2016	17.06.2016	05.07.2016	30.06.2016		
30133018	215	30320013	f	Cre:Cre+; Pink1:flox/flox	29.07.2014	07.10.2015	14.5	14.10.2015	14.7	04.11.2015	15.4					18.03.2016	19.93		20.06.2016	06.07.2016	30.06.2016		
30133502	223	30320014	f	Cre:Cre+; Pink1:flox/flox	31.07.2014	07.10.2015	14.4	14.10.2015	14.7	04.11.2015	15.4					18.03.2016	19.87	01.06.2016	20.06.2016	05.07.2016	04.07.2016		
30133517	238	30320015	f	Cre:Cre+; Pink1:flox/flox	31.07.2014	07.10.2015	14.4	14.10.2015	14.7	05.11.2015	15.4					18.03.2016	19.87	01.06.2016	20.06.2016	05.07.2016	30.06.2016		
30133555	283	30320016	f	Cre:Cre+; Pink1:flox/flox	01.08.2014	07.10.2015	14.4	14.10.2015	14.6	05.11.2015	15.4					18.03.2016	19.83	01.06.2016	27.06.2016	04.07.2016	01.07.2016		
30133769	357	30320017	f	Cre:Cre+; Pink1:flox/flox	03.08.2014	07.10.2015	14.3	14.10.2015	14.6	05.11.2015	15.3					18.03.2016	19.77	01.06.2016	27.06.2016	04.07.2016	01.07.2016		
30134065	370	30320018	f	Cre:Cre+; Pink1:flox/flox	07.08.2014																		
30135109	387	30320019	f	Cre:Cre+; Pink1:flox/flox	24.08.2014	07.10.2015	13.6	14.10.2015	13.9	05.11.2015	14.6					18.03.2016	19.07	01.06.2016	16.06.2016	06.07.2016	01.07.2016		
30135124	391	30320020	f	Cre:Cre+; Pink1:flox/flox	24.08.2014	07.10.2015	13.6	14.10.2015	13.9	05.11.2015	14.6												
	577	30146744	f	Cre:Cre+; Pink1:flox/flox	08.02.2015											08.09.2016	19.27					06.10.2016	
	655	30157188	f	Cre:Cre+; Pink1:flox/flox	12.06.2015											08.09.2016	15.13					06.10.2016	
30132708	148	30319996	f	Cre:Cre-; Pink1:flox/flox	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00	01.06.2016	17.06.2016	06.07.2016	01.07.2016		
30132755	174	30319997	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	07.10.2015	14.5	14.10.2015	14.8	04.11.2015	15.5												
30132768	187	30319998	f	Cre:Cre-; Pink1:flox/flox	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	20.00	01.06.2016	20.06.2016	05.07.2016	01.07.2016		
30132981	199	30319999	f	Cre:Cre-; Pink1:flox/flox	27.07.2014	07.10.2015	14.6	14.10.2015	14.8	04.11.2015	15.5												
30132996	203	30320000	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	07.10.2015	14.5	14.10.2015	14.8	04.11.2015	15.5					18.03.2016	19.97		16.06.2016	05.07.2016	01.07.2016		
30132997	204	30320001	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	07.10.2015	14.5	14.10.2015	14.8	05.11.2015	15.5					18.03.2016	19.97	01.06.2016	17.06.2016	05.07.2016	05.08.2016		
30133016	213	30320002	f	Cre:Cre-; Pink1:flox/flox	29.07.2014	07.10.2015	14.5	14.10.2015	14.7	05.11.2015	15.5					18.03.2016	19.93	01.06.2016	20.06.2016	05.07.2016	30.06.2016		
30133510	231	30320003	f	Cre:Cre-; Pink1:flox/flox	31.07.2014	07.10.2015	14.4	14.10.2015	14.7	05.11.2015	15.4												
30133527	257	30320004	f	Cre:Cre-; Pink1:flox/flox	31.07.2014	07.10.2015	14.4	14.10.2015	14.7	05.11.2015	15.4					18.03.2016	19.87	01.06.2016	20.06.2016	06.07.2016	04.07.2016		
30133528	258	30320005	f	Cre:Cre-; Pink1:flox/flox	31.07.2014	07.10.2015	14.4	14.10.2015	14.7	05.11.2015	15.4					18.03.2016	19.87	01.06.2016	04.07.2016	06.07.2016	30.06.2016		
30135126	393	30320006	f	Cre:Cre-; Pink1:flox/flox	24.08.2014	07.10.2015	13.6	14.10.2015	13.9	05.11.2015	14.6					18.03.2016	19.07	01.06.2016	20.06.2016	06.07.2016	01.07.2016		
30135144	401	30320007	f	Cre:Cre-; Pink1:flox/flox	23.08.2014																		
30133000	207	30320008	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	07.10.2015	14.5	14.10.2015	14.8	06.11.2015	15.5												
30133001	208	30320009	f	Cre:Cre-; Pink1:flox/flox	28.07.2014	07.10.2015	14.5	14.10.2015	14.8	06.11.2015	15.5					18.03.2016	19.97						
	492	30146303	f	Cre:Cre-; Pink1:flox/flox	31.01.2015											08.09.2016	19.53					06.10.2016	
	576	30146743	f	Cre:Cre-; Pink1:flox/flox	07.02.2015											08.09.2016	19.30					06.10.2016	

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition, TH = immunohistochemical staining of TH^+ cells, 5-HT = immunohistochemical staining of $5\text{-}HT^+$ cells, HPLC = quantification of neurotransmitter content via HPLC, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, date est. = estimated date. The age is expressed in months.

Table A5: Overview of analysed young male Pink1 x DAT mice of the cohort 1

male Pink1 x DAT, cohort 1																
						young										
						OF		ASR/PPI		Catwalk		Olfaction				
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age	
30139019	119	30327195	m	Cre:Cre+; Pink1:wt/wt	06.10.2014	16.02.2015	4.4	23.02.2015	4.7	31.03.2015	5.9	22.04.2015	6.6	20.05.2015	7.5	
30139298	124	30327196	m	Cre:Cre+; Pink1:wt/wt	06.10.2014	16.02.2015	4.4	23.02.2015	4.7	31.03.2015	5.9	22.04.2015	6.6	20.05.2015	7.5	
30139375	146	30327199	m	Cre:Cre+; Pink1:wt/wt	07.10.2014	16.02.2015	4.4	23.02.2015	4.6	31.03.2015	5.8	22.04.2015	6.6	20.05.2015	7.5	
30139468	168	30327201	m	Cre:Cre+; Pink1:wt/wt	09.10.2014	16.02.2015	4.3	23.02.2015	4.6	01.04.2015	5.8	22.04.2015	6.5	20.05.2015	7.4	
30139385	150	30327204	m	Cre:Cre+; Pink1:wt/wt	07.10.2014	16.02.2015	4.4	23.02.2015	4.6	01.04.2015	5.9					
30139585	195	30327207	m	Cre:Cre+; Pink1:wt/wt	09.10.2014	16.02.2015	4.3	23.02.2015	4.6	01.04.2015	5.8	22.04.2015	6.5	20.05.2015	7.4	
30140012	303	30327209	m	Cre:Cre+; Pink1:wt/wt	16.10.2014	16.02.2015	4.1	23.02.2015	4.3	01.04.2015	5.6	22.04.2015	6.3	20.05.2015	7.2	
30140014	305	30327210	m	Cre:Cre+; Pink1:wt/wt	16.10.2014	16.02.2015	4.1	23.02.2015	4.3	01.04.2015	5.6	22.04.2015	6.3	20.05.2015	7.2	
30139617	208	30327211	m	Cre:Cre+; Pink1:wt/wt	10.10.2014	16.02.2015	4.3	23.02.2015	4.5	02.04.2015	5.8	22.04.2015	6.5	20.05.2015	7.4	
30139723	282	30327226	m	Cre:Cre+; Pink1:wt/wt	13.10.2014	16.02.2015	4.2	23.02.2015	4.4	02.04.2015	5.7					
30139728	287	30327228	m	Cre:Cre+; Pink1:wt/wt	10.10.2014	16.02.2015	4.3	23.02.2015	4.5	02.04.2015	5.8					
30140161	351	30327233	m	Cre:Cre+; Pink1:wt/wt	15.10.2014	16.02.2015	4.1	23.02.2015	4.4	02.04.2015	5.6					
30139633	216	30327192	m	Cre:Cre+; Pink1:flox/flox	10.10.2014	16.02.2015	4.3	23.02.2015	4.5	31.03.2015	5.7	22.04.2015	6.5	20.05.2015	7.4	
30140046	317	30327193	m	Cre:Cre+; Pink1:flox/flox	17.10.2014	16.02.2015	4.1	23.02.2015	4.3	31.03.2015	5.5	22.04.2015	6.2	20.05.2015	7.2	
30139423	162	30327200	m	Cre:Cre+; Pink1:flox/flox	09.10.2014	16.02.2015	4.3	23.02.2015	4.6	31.03.2015	5.8	22.04.2015	6.5	20.05.2015	7.4	
30139480	180	30327202	m	Cre:Cre+; Pink1:flox/flox	09.10.2014	16.02.2015	4.3	23.02.2015	4.6	31.03.2015	5.8					
30139606	201	30327208	m	Cre:Cre+; Pink1:flox/flox	10.10.2014	16.02.2015	4.3	23.02.2015	4.5	01.04.2015	5.8	22.04.2015	6.5	20.05.2015	7.4	
30140048	319	30327216	m	Cre:Cre+; Pink1:flox/flox	17.10.2014	16.02.2015	4.1	23.02.2015	4.3	01.04.2015	5.5	22.04.2015	6.2	20.05.2015	7.2	
30140053	324	30327217	m	Cre:Cre+; Pink1:flox/flox	16.10.2014	16.02.2015	4.1	23.02.2015	4.3	02.04.2015	5.6	22.04.2015	6.3	20.05.2015	7.2	
30140054	325	30327218	m	Cre:Cre+; Pink1:flox/flox	16.10.2014	16.02.2015	4.1	23.02.2015	4.3	01.04.2015	5.6	22.04.2015	6.3	20.05.2015	7.2	
30139717	278	30327225	m	Cre:Cre+; Pink1:flox/flox	13.10.2014	16.02.2015	4.2	23.02.2015	4.4	02.04.2015	5.7					
30139730	289	30327227	m	Cre:Cre+; Pink1:flox/flox	10.10.2014	16.02.2015	4.3	23.02.2015	4.5	02.04.2015	5.8					
30140162	352	30327234	m	Cre:Cre+; Pink1:flox/flox	15.10.2014	16.02.2015	4.1	23.02.2015	4.4	02.04.2015	5.6	22.04.2015	6.3	20.05.2015	7.2	
30139618	209	30327190	m	Cre:Cre-; Pink1:flox/flox	10.10.2014	16.02.2015	4.3	23.02.2015	4.5	31.03.2015	5.7	22.04.2015	6.5	20.05.2015	7.4	
30139631	214	30327191	m	Cre:Cre-; Pink1:flox/flox	10.10.2014	16.02.2015	4.3	23.02.2015	4.5	31.03.2015	5.7	22.04.2015	6.5	20.05.2015	7.4	
30139299	125	30327197	m	Cre:Cre-; Pink1:flox/flox	06.10.2014	16.02.2015	4.4	23.02.2015	4.7	31.03.2015	5.9	22.04.2015	6.6	20.05.2015	7.5	
30139533	185	30327203	m	Cre:Cre-; Pink1:flox/flox	09.10.2014	16.02.2015	4.3	23.02.2015	4.6	31.03.2015	5.8					
30139672	245	30327213	m	Cre:Cre-; Pink1:flox/flox	13.10.2014	16.02.2015	4.2	23.02.2015	4.4	01.04.2015	5.7	22.04.2015	6.4	20.05.2015	7.3	
30139674	247	30327214	m	Cre:Cre-; Pink1:flox/flox	13.10.2014	16.02.2015	4.2	23.02.2015	4.4	02.04.2015	5.7					
30139675	248	30327215	m	Cre:Cre-; Pink1:flox/flox	13.10.2014	16.02.2015	4.2	23.02.2015	4.4	01.04.2015	5.7	22.04.2015	6.4	20.05.2015	7.3	
30140055	326	30327219	m	Cre:Cre-; Pink1:flox/flox	16.10.2014	16.02.2015	4.1	23.02.2015	4.3	01.04.2015	5.6	22.04.2015	6.3	20.05.2015	7.2	
30139693	257	30327222	m	Cre:Cre-; Pink1:flox/flox	12.10.2014	16.02.2015	4.2	23.02.2015	4.5	02.04.2015	5.7	22.04.2015	6.4	20.05.2015	7.3	
30139716	277	30327224	m	Cre:Cre-; Pink1:flox/flox	13.10.2014	16.02.2015	4.2	23.02.2015	4.4	02.04.2015	5.7					
30140043	314	30327231	m	Cre:Cre-; Pink1:flox/flox	16.10.2014	16.02.2015	4.1	23.02.2015	4.3	02.04.2015	5.6					
30140160	350	30327232	m	Cre:Cre-; Pink1:flox/flox	15.10.2014	16.02.2015	4.1	23.02.2015	4.4	02.04.2015	5.6	22.04.2015	6.3	20.05.2015	7.2	

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. Some animals, tested in olfaction testing, could not be considered for the analysis (red colour). OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition. The age is expressed in months.

Table A6: Overview of analysed mid-aged male Pink1 x DAT mice of the cohort 1

male Pink1 x DAT, cohort 1																										
						mid aged																				
						OF		ASR/PPI		Catwalk		Olfaction				prep		TH	HPLC			5-HT				
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age	date	age	date est.	date OB	date STR	date VM	date				
30139019	119	30327195	m	Cre:Cre+; Pink1:wt/wt	06.10.2014	12.01.2016	15.4	19.01.2016	15.7	01.02.2016	16.1	14.03.2016	17.5	08.04.2016	18.3	20.04.2016	18.73	15.06.2016	14.07.2016	28.07.2016	21.07.2016						
30139298	124	30327196	m	Cre:Cre+; Pink1:wt/wt	06.10.2014	12.01.2016	15.4	19.01.2016	15.7	01.02.2016	16.1	14.03.2016	17.5	08.04.2016	18.3	20.04.2016	18.73	15.06.2016	13.07.2016	29.07.2016	21.07.2016						
30139375	146	30327199	m	Cre:Cre+; Pink1:wt/wt	07.10.2014	12.01.2016	15.4	20.01.2016	15.7	02.02.2016	16.1	14.03.2016	17.5	08.04.2016	18.3	20.04.2016	18.70	15.06.2016	13.07.2016	27.07.2016	15.07.2016						
30139468	168	30327201	m	Cre:Cre+; Pink1:wt/wt	09.10.2014	12.01.2016	15.3	19.01.2016	15.6	01.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.63	15.06.2016	14.07.2016	28.07.2016	22.07.2016						
30139385	150	30327204	m	Cre:Cre+; Pink1:wt/wt	07.10.2014	12.01.2016	15.4	19.01.2016	15.6	01.02.2016	16.1					20.04.2016	18.70										
30139585	195	30327207	m	Cre:Cre+; Pink1:wt/wt	09.10.2014	12.01.2016	15.3	19.01.2016	15.6	01.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.63	15.06.2016	14.07.2016	28.07.2016	21.07.2016						
30140012	303	30327209	m	Cre:Cre+; Pink1:wt/wt	16.10.2014	12.01.2016	15.1	20.01.2016	15.4	01.02.2016	15.8	14.03.2016	17.2	08.04.2016	18.0	20.04.2016	18.40	15.06.2016	13.07.2016	29.07.2016	21.07.2016						
30140014	305	30327210	m	Cre:Cre+; Pink1:wt/wt	16.10.2014	12.01.2016	15.1	20.01.2016	15.4	02.02.2016	15.8	14.03.2016	17.2	08.04.2016	18.0	20.04.2016	18.40	15.06.2016									
30139617	208	30327211	m	Cre:Cre+; Pink1:wt/wt	10.10.2014	12.01.2016	15.3	20.01.2016	15.6	02.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.60										
30139723	282	30327226	m	Cre:Cre+; Pink1:wt/wt	13.10.2014	12.01.2016	15.2	20.01.2016	15.5	02.02.2016	15.9					22.03.2016	17.53		07.07.2016	28.07.2016	12.08.2016						
30139728	287	30327228	m	Cre:Cre+; Pink1:wt/wt	10.10.2014	12.01.2016	15.3	20.01.2016	15.6	02.02.2016	16.0					22.03.2016	17.63	15.06.2016	07.07.2016	27.07.2016	15.07.2016						
30140161	351	30327233	m	Cre:Cre+; Pink1:wt/wt	15.10.2014																	01.12.2016				
30139633	216	30327192	m	Cre:Cre+; Pink1:flox/flox	10.10.2014	12.01.2016	15.3	19.01.2016	15.5	01.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.60	15.06.2016	14.07.2016	27.07,2016	22.07.2016	01.12.2016					
30140046	317	30327193	m	Cre:Cre+; Pink1:flox/flox	17.10.2014	12.01.2016	15.1	19.01.2016	15.3	01.02.2016	15.7	14.03.2016	17.1	08.04.2016	18.0	20.04.2016	18.37	15.06.2016	07.07.2016	28.07.2016	22.07.2016						
30139423	162	30327200	m	Cre:Cre+; Pink1:flox/flox	09.10.2014	12.01.2016	15.3	19.01.2016	15.6	01.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.63	15.06.2016	13.07.2016	29.07.2016	21.07.2016	01.12.2016					
30139480	180	30327202	m	Cre:Cre+; Pink1:flox/flox	09.10.2014	12.01.2016	15.3	19.01.2016	15.6	01.02.2016	16.0					22.03.2016	17.67	15.06.2016	07.07.2016	27.07.2016	21.07.2016						
30139606	201	30327208	m	Cre:Cre+; Pink1:flox/flox	10.10.2014	12.01.2016	15.3	20.01.2016	15.6	02.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.60	15.06.2016	13.07.2016	28.07.2016	15.07.2016						
30140048	319	30327216	m	Cre:Cre+; Pink1:flox/flox	17.10.2014	12.01.2016	15.1	20.01.2016	15.3	02.02.2016	15.8	14.03.2016	17.1	08.04.2016	18.0	20.04.2016	18.37										
30140053	324	30327217	m	Cre:Cre+; Pink1:flox/flox	16.10.2014	12.01.2016	15.1	20.01.2016	15.4	02.02.2016	15.8	14.03.2016	17.2	08.04.2016	18.0	20.04.2016	18.40	15.06.2016	14.07.2016	29.07.2016	22.07.2016						
30140054	325	30327218	m	Cre:Cre+; Pink1:flox/flox	16.10.2014																					
30139717	278	30327225	m	Cre:Cre+; Pink1:flox/flox	13.10.2014	12.01.2016	15.2	19.01.2016	15.4	01.02.2016	15.9					22.03.2016	17.53	15.06.2016	13.07.2016	27.07.216	12.08.2016						
30139730	289	30327227	m	Cre:Cre+; Pink1:flox/flox	10.10.2014	12.01.2016	15.3	20.01.2016	15.6	01.02.2016	16.0					22.03.2016	17.63	15.06.2016	07.07.2016	29.07.2016	15.07.289						
30140162	352	30327234	m	Cre:Cre+; Pink1:flox/flox	15.10.2014	12.01.2016	15.1	20.01.2016	15.4	02.02.2016	15.8	14.03.2016	17.2	08.04.2016	18.0	20.04.2016	18.43										
30139618	209	30327190	m	Cre:Cre-; Pink1:flox/flox	10.10.2014	12.01.2016	15.3	19.01.2016	15.5	01.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.60	15.06.2016	14.07.2016	28.07.2016	15.07.2016	01.12.2016					
30139631	214	30327191	m	Cre:Cre-; Pink1:flox/flox	10.10.2014	12.01.2016	15.3	19.01.2016	15.5	01.02.2016	16.0	14.03.2016	17.4	08.04.2016	18.2	20.04.2016	18.60	15.06.2016	14.07.2016	28.07.2016	21.07.2016	01.12.2016					
30139299	125	30327197	m	Cre:Cre-; Pink1:flox/flox	06.10.2014	12.01.2016	15.4	19.01.2016	15.7	01.02.2016	16.1	14.03.2016	17.5	08.04.2016	18.3	20.04.2016	18.73	15.06.2016	13.07.2016	27.07.2016	21.07.2016						
30139533	185	30327203	m	Cre:Cre-; Pink1:flox/flox	09.10.2014	12.01.2016	15.3	19.01.2016	15.6	01.02.2016	16.0					22.03.2016	17.67	15.06.2016	07.07.2016	28.07.2016	12.08.2016						
30139672	245	30327213	m	Cre:Cre-; Pink1:flox/flox	13.10.2014	12.01.2016	15.2	20.01.2016	15.5	01.02.2016	15.9	14.03.2016	17.3	08.04.2016	18.1	20.04.2016	18.50		13.07.2016	29.07.2016	21.07.2016						
30139674	247	30327214	m	Cre:Cre-; Pink1:flox/flox	13.10.2014																					
30139675	248	30327215	m	Cre:Cre-; Pink1:flox/flox	13.10.2014																					
30140055	326	30327219	m	Cre:Cre-; Pink1:flox/flox	16.10.2014	12.01.2016	15.1	20.01.2016	15.4	02.02.2016	15.8	14.03.2016	17.2	08.04.2016	18.0	20.04.2016	18.40	15.06.2016									
30139693	257	30327222	m	Cre:Cre-; Pink1:flox/flox	12.10.2014	12.01.2016	15.2	20.01.2016	15.5	02.02.2016	15.9	14.03.2016	17.3	08.04.2016	18.1	20.04.2016	18.53	15.06.2016									
30139716	277	30327224	m	Cre:Cre-; Pink1:flox/flox	13.10.2014	12.01.2016	15.2	20.01.2016	15.5	02.02.2016	15.9					22.03.2016	17.53		07.07.2016	28.07.2016	15.07.2016						
30140043	314	30327231	m	Cre:Cre-; Pink1:flox/flox	16.10.2014	12.01.2016	15.1	20.01.2016	15.4	02.02.2016	15.8					22.03.2016	17.43	15.06.2016	13.07.2016	28.07.2016	15.07.2016	01.12.2016					
30140160	350	30327232	m	Cre:Cre-; Pink1:flox/flox	15.10.2014	12.01.2016	15.1	19.01.2016	15.4	01.02.2016	15.8	14.03.2016	17.2	08.04.2016	18.0	20.04.2016	18.43	15.06.2016	04.08.2016	27.07.2016	21.07.2016						

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. Some animals, tested in olfaction testing, could not be considered for the analysis (red colour). OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition, TH = immunohistochemical staining of TH^+ cells, 5-HT = immunohistochemical staining of $5\text{-}HT^+$ cells, HPLC = quantification of neurotransmitter content via HPLC, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, date est. = estimated date. The age is expressed in months.

Table A7: Overview of analysed young female Pink1 x DAT mice of the cohort 1

female Pink1 x DAT, cohort 1															
						young									
						OF		ASR/PPI		Catwalk		Olfaction			
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age
30139647	224	30327235	f	Cre:Cre+; Pink1:wt/wt	11.10.2014	17.02.2015	4.3	24.02.2015	4.5	07.04.2015	5.9				
30139649	226	30327236	f	Cre:Cre+; Pink1:wt/wt	11.10.2014	17.02.2015	4.3	24.02.2015	4.5	07.04.2015	5.9				
30140171	361	30327237	f	Cre:Cre+; Pink1:wt/wt	23.10.2014	17.02.2015	3.9	24.02.2015	4.1	07.04.2015	5.5				
30139017	117	30327241	f	Cre:Cre+; Pink1:wt/wt	06.10.2014	17.02.2015	4.5	23.02.2015	4.7	02.04.2015	5.9				
30139362	133	30327244	f	Cre:Cre+; Pink1:wt/wt	07.10.2014	17.02.2015	4.4	24.02.2015	4.7	07.04.2015	6.1				
30139479	179	30327252	f	Cre:Cre+; Pink1:wt/wt	09.10.2014	17.02.2015	4.4	24.02.2015	4.6	08.04.2015	6.0				
30140018	309	30327260	f	Cre:Cre+; Pink1:wt/wt	16.10.2014	17.02.2015	4.1	24.02.2015	4.4	08.04.2015	5.8				
30139652	229	30327264	f	Cre:Cre+; Pink1:wt/wt	12.10.2014	17.02.2015	4.3	24.02.2015	4.5	08.04.2015	5.9				
30139719	280	30327274	f	Cre:Cre+; Pink1:wt/wt	13.10.2014	17.02.2015	4.2	24.02.2015	4.5	08.04.2015	5.9				
30139734	293	30327277	f	Cre:Cre+; Pink1:wt/wt	10.10.2014	17.02.2015	4.3	24.02.2015	4.6	08.04.2015	6.0				
30139021	121	30327243	f	Cre:Cre+; Pink1:flox/flox	06.10.2014	17.02.2015	4.5	23.02.2015	4.7	02.04.2015	5.9				
30139371	142	30327246	f	Cre:Cre+; Pink1:flox/flox	07.10.2014	17.02.2015	4.4	24.02.2015	4.7	07.04.2015	6.1				
30139482	182	30327253	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	17.02.2015	4.4	24.02.2015	4.6	07.04.2015	6.0				
30139484	184	30327254	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	17.02.2015	4.4	24.02.2015	4.6	07.04.2015	6.0				
30139537	189	30327255	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	17.02.2015	4.4	24.02.2015	4.6	07.04.2015	6.0				
30139424	163	30327256	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	17.02.2015	4.4	24.02.2015	4.6	07.04.2015	6.0				
30139471	171	30327257	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	17.02.2015	4.4	24.02.2015	4.6	08.04.2015	6.0				
30139610	205	30327259	f	Cre:Cre+; Pink1:flox/flox	10.10.2014	17.02.2015	4.3	24.02.2015	4.6	08.04.2015	6.0				
30139654	231	30327265	f	Cre:Cre+; Pink1:flox/flox	12.10.2014	17.02.2015	4.3	24.02.2015	4.5	08.04.2015	5.9				
30139655	232	30327266	f	Cre:Cre+; Pink1:flox/flox	12.10.2014	17.02.2015	4.3	24.02.2015	4.5	08.04.2015	5.9				
30139680	253	30327270	f	Cre:Cre+; Pink1:flox/flox	13.10.2014	17.02.2015	4.2	24.02.2015	4.5	08.04.2015	5.9				
30139733	292	30327276	f	Cre:Cre+; Pink1:flox/flox	10.10.2014	17.02.2015	4.3	24.02.2015	4.6	08.04.2015	6.0				
30139013	113	30327239	f	Cre:Cre-; Pink1:flox/flox	06.10.2014	17.02.2015	4.5	23.02.2015	4.7	02.04.2015	5.9				
30139016	116	30327240	f	Cre:Cre-; Pink1:flox/flox	06.10.2014	17.02.2015	4.5	23.02.2015	4.7	07.04.2015	6.1				
30139372	143	30327247	f	Cre:Cre-; Pink1:flox/flox	07.10.2014	17.02.2015	4.4	24.02.2015	4.7	07.04.2015	6.1				
30139373	144	30327248	f	Cre:Cre-; Pink1:flox/flox	07.10.2014	17.02.2015	4.4	24.02.2015	4.7	07.04.2015	6.1				
30139377	148	30327249	f	Cre:Cre-; Pink1:flox/flox	07.10.2014	17.02.2015	4.4	24.02.2015	4.7	07.04.2015	6.1				
30139476	176	30327251	f	Cre:Cre-; Pink1:flox/flox	09.10.2014	17.02.2015	4.4	24.02.2015	4.6	07.04.2015	6.0				
30139638	221	30327262	f	Cre:Cre-; Pink1:flox/flox	10.10.2014	17.02.2015	4.3	24.02.2015	4.6	08.04.2015	6.0				
30139665	242	30327267	f	Cre:Cre-; Pink1:flox/flox	12.10.2014	17.02.2015	4.3	24.02.2015	4.5	08.04.2015	5.9				
30139683	256	30327268	f	Cre:Cre-; Pink1:flox/flox	13.10.2014	17.02.2015	4.2	24.02.2015	4.5	08.04.2015	5.9				
30139678	251	30327269	f	Cre:Cre-; Pink1:flox/flox	13.10.2014	17.02.2015	4.2	24.02.2015	4.5	08.04.2015	5.9				
30139682	255	30327271	f	Cre:Cre-; Pink1:flox/flox	13.10.2014	17.02.2015	4.2	24.02.2015	4.5	08.04.2015	5.9				
30139699	263	30327272	f	Cre:Cre-; Pink1:flox/flox	12.10.2014	17.02.2015	4.3	24.02.2015	4.5	08.04.2015	5.9				

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. Some movies of CatWalk testing (labelled in red colour) could not be analysed and were not considered for the analysis. OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition. The age is expressed in months.

Table A8: Overview of analysed mid-aged female Pink1 x DAT mice of the cohort 1

female Pink1 x DAT, cohort 1																									
						mid aged																			
						OF		ASR/PPI		Catwalk		Olfaction				prep		TH	HPLC			5-HT	
ID C-Streifen	ear nr.	ID GMC	sex	genotype	born	date	age	date	age	date	age	begin date	age	end date	age	date	age	date est.	date OB	date STR	date VM	date	
30139647	224	30327235	f	Cre:Cre+; Pink1:wt/wt	11.10.2014	13.01.2016	15.3	19.01.2016	15.5	03.02.2016	16.0					21.03.2016	17.57	15.06.2016	14.07.2016	27.07.2016	15.07.2016		
30139649	226	30327236	f	Cre:Cre+; Pink1:wt/wt	11.10.2014	13.01.2016	15.3	19.01.2016	15.5	03.02.2016	16.0					21.03.2016	17.57	15.06.2016	15.07.2016	27.07.2016	15.07.2016		
30140171	361	30327237	f	Cre:Cre+; Pink1:wt/wt	23.10.2014	13.01.2016	14.9	19.01.2016	15.1	03.02.2016	15.6					21.03.2016	17.17		07.07.2016	27.07.2016	22.07.2016		
30139017	117	30327241	f	Cre:Cre+; Pink1:wt/wt	06.10.2014	13.01.2016	15.5	19.01.2016	15.7	03.02.2016	16.2					21.03.2016	17.73	15.06.2016	13.07.2016	28.07.2016	22.07.2016		
30139362	133	30327244	f	Cre:Cre+; Pink1:wt/wt	07.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.70	15.06.2016	07.07.2016	29.07.2016	21.07.2016		
30139479	179	30327252	f	Cre:Cre+; Pink1:wt/wt	09.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.63	15.06.2016	07.07.2016	29.07.2016	21.07.2016		
30140018	309	30327260	f	Cre:Cre+; Pink1:wt/wt	16.10.2014	13.01.2016	15.1	19.01.2016	15.3	04.02.2016	15.9					21.03.2016	17.40	15.06.2016	07.07.2016	27.07.2016	12.08.2016		
30139652	229	30327264	f	Cre:Cre+; Pink1:wt/wt	12.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.0					21.03.2016	17.53						
30139719	280	30327274	f	Cre:Cre+; Pink1:wt/wt	13.10.2014	13.01.2016	15.2	19.01.2016	15.4	04.02.2016	16.0					21.03.2016	17.50	15.06.2016	14.07.2016	28.08.2016	21.07.2016		
30139734	293	30327277	f	Cre:Cre+; Pink1:wt/wt	10.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.1					21.03.2016	17.60	15.06.2016				01.12.2016	
30139021	121	30327243	f	Cre:Cre+; Pink1:flox/flox	06.10.2014	13.01.2016	15.5	19.01.2016	15.7	03.02.2016	16.2					21.03.2016	17.73		07.07.2016	28.07.2016	22.07.2016	01.12.2016	
30139371	142	30327246	f	Cre:Cre+; Pink1:flox/flox	07.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.70	15.06.2016	13.07.2016	28.07.2016	21.07.2016	01.12.2016	
30139482	182	30327253	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.63	15.06.2016	13.07.2016	27.07.2016	21.07.2016		
30139484	184	30327254	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.63		14.07.2016	27.07.2016	15.07.2016		
30139537	189	30327255	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.63	15.06.2016	14.07.2016	28.07.2016	15.07.2016		
30139424	163	30327256	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.63	15.06.2016	14.07.2016	28.07.2016	21.07.2016		
30139471	171	30327257	f	Cre:Cre+; Pink1:flox/flox	09.10.2014	13.01.2016	15.4	19.01.2016	15.6	04.02.2016	16.1					21.03.2016	17.63	15.06.2016	13.07.2016	27.07.2016	21.07.2016		
30139610	205	30327259	f	Cre:Cre+; Pink1:flox/flox	10.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.1					21.03.2016	17.60	15.06.2016	07.07.2016	28.07.2016	22.07.2016		
30139654	231	30327265	f	Cre:Cre+; Pink1:flox/flox	12.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.0					21.03.2016	17.53	15.06.2016					
30139655	232	30327266	f	Cre:Cre+; Pink1:flox/flox	12.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.0					22.03.2016	17.57						
30139680	253	30327270	f	Cre:Cre+; Pink1:flox/flox	13.10.2014																		
30139733	292	30327276	f	Cre:Cre+; Pink1:flox/flox	10.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.1												
30139013	113	30327239	f	Cre:Cre-; Pink1:flox/flox	06.10.2014	13.01.2016	15.5	19.01.2016	15.7	03.02.2016	16.2					21.03.2016	17.73		13.07.2016	28.07.2016	22.07.2016	01.12.2016	
30139016	116	30327240	f	Cre:Cre-; Pink1:flox/flox	06.10.2014	13.01.2016	15.5	19.01.2016	15.7	03.02.2016	16.2					21.03.2016	17.73	15.06.2016	07.07.2016	27.07.2016	15.07.2016	01.12.2016	
30139372	143	30327247	f	Cre:Cre-; Pink1:flox/flox	07.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.70	15.06.2016	14.07.2016	28.07.2016	21.07.2016	01.12.2016	
30139373	144	30327248	f	Cre:Cre-; Pink1:flox/flox	07.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.70	15.06.2016	13.07.2016	27.07.2016	21.07.2016		
30139377	148	30327249	f	Cre:Cre-; Pink1:flox/flox	07.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					22.03.2016	17.73		14.07.2016	27.07.2016	15.07.2016		
30139476	176	30327251	f	Cre:Cre-; Pink1:flox/flox	09.10.2014	13.01.2016	15.4	19.01.2016	15.6	03.02.2016	16.1					21.03.2016	17.63	15.06.2016	11.07.2016	27.07.2016	21.07.2016		
30139638	221	30327262	f	Cre:Cre-; Pink1:flox/flox	10.10.2014	13.01.2016	15.3	19.01.2016	15.5	03.02.2016	16.0					21.03.2016	17.60	15.06.2016	04.08.2016	29.07.2016	21.07.2016		
30139665	242	30327267	f	Cre:Cre-; Pink1:flox/flox	12.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.0					21.03.2016	17.53	15.06.2016	04.08.2016	27.07.2016	12.08.2016		
30139683	256	30327268	f	Cre:Cre-; Pink1:flox/flox	13.10.2014	13.01.2016	15.2	19.01.2016	15.4	04.02.2016	16.0					21.03.2016	17.50	15.06.2016					
30139678	251	30327269	f	Cre:Cre-; Pink1:flox/flox	13.10.2014	13.01.2016	15.2	19.01.2016	15.4	04.02.2016	16.0					21.03.2016	17.50						
30139682	255	30327271	f	Cre:Cre-; Pink1:flox/flox	13.10.2014	13.01.2016	15.2	19.01.2016	15.4	04.02.2016	16.0												
30139699	263	30327272	f	Cre:Cre-; Pink1:flox/flox	12.10.2014	13.01.2016	15.3	19.01.2016	15.5	04.02.2016	16.0												

Mice were born in the mouse facility “C-Streifen” and were tested in the mouse facility “GMC”. OF = open field, ASR = acoustic startle reflex, PPI = prepulse inhibition, TH = immunohistochemical staining of TH^+ cells, 5-HT = immunohistochemical staining of 5-HT^+ cells, HPLC = quantification of neurotransmitter content via HPLC, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, date est. = estimated date. The age is expressed in months.

Table A9: Overview of analysed Pink1 x Pet mice of the cohort 2

Pink1 x Pet, cohort 2
young

ear nr.	ID C-Streifen	sex	genotype	born	prep	age	TH date est.	TH date OB	HPLC date STR	HPLC date VM
745	30157395	m	Cre:Cre+; Pink1:wt/wt	14.06.2015	30.09.2015	3.60	02.11.2015	14.01.2016	20.01.2016	03.12.2015
746	30157396	m	Cre:Cre+; Pink1:wt/wt	14.06.2015	30.09.2015	3.60	02.11.2015	15.01.2016	25.01.2016	14.12.2015
775	30157425	m	Cre:Cre+; Pink1:wt/wt	15.06.2015	30.09.2015	3.57	02.11.2015	15.01.2016	20.01.2016	14.12.2015
937	30161163	m	Cre:Cre+; Pink1:wt/wt	18.07.2015	19.11.2015	4.13	02.11.2015	18.01.2016	28.01.2016	16.12.2015
948	30161820	m	Cre:Cre+; Pink1:wt/wt	18.07.2015	19.11.2015	4.13	02.11.2015	14.01.2016		
953	30161825	m	Cre:Cre+; Pink1:wt/wt	18.07.2015	19.11.2015	4.13	02.11.2015			
988	30161942	m	Cre:Cre+; Pink1:wt/wt	25.07.2015	19.11.2015	3.90	02.11.2015	18.01.2016	25.01.2016	15.12.2015
909	30159757	m	Cre:Cre+; Pink1:wt/wt	08.07.2015	02.11.2015	3.90			29.01.2016	14.12.2015
917	30159920	m	Cre:Cre+; Pink1:wt/wt	08.07.2015	02.11.2015	3.90		13.01.2016	20.01.2016	10.12.2015
980	30161927	m	Cre:Cre+; Pink1:wt/wt	25.07.2015	19.11.2015	3.90		19.01.2016	27.01.2016	16.12.2015
671	30157204	m	Cre:Cre+; Pink1:flox/flox	13.06.2015	30.09.2015	3.63	02.11.2015	14.01.2016	21.01.2016	03.12.2015
679	30157212	m	Cre:Cre+; Pink1:flox/flox	13.06.2015	30.09.2015	3.63	02.11.2015	15.01.2016		14.12.2015
713	30157363	m	Cre:Cre+; Pink1:flox/flox	13.06.2015	30.09.2015	3.63			28.01.2016	14.12.2015
739	30157389	m	Cre:Cre+; Pink1:flox/flox	14.06.2015	30.09.2015	3.60		18.01.2016	27.01.2016	15.12.2015
752	30157402	m	Cre:Cre+; Pink1:flox/flox	14.06.2015	30.09.2015	3.60	02.11.2015	13.01.2016		10.12.2015
753	30157403	m	Cre:Cre+; Pink1:flox/flox	14.06.2015	30.09.2015	3.60	02.11.2015	14.01.2016	21.01.2016	10.12.2015
755	30157405	m	Cre:Cre+; Pink1:flox/flox	14.06.2015	02.10.2015	3.67		15.01.2016	27.01.2016	16.12.2015
765	30157415	m	Cre:Cre+; Pink1:flox/flox	15.06.2015	02.10.2015	3.63	02.11.2015	15.01.2016	25.01.2016	17.12.2016
789	30157439	m	Cre:Cre+; Pink1:flox/flox	15.06.2015	02.10.2015	3.63	02.11.2015		29.01.2016	
795	30157445	m	Cre:Cre+; Pink1:flox/flox	15.06.2015	02.10.2015	3.63	02.11.2015	18.01.2016	21.01.2016	
670	30157203	m	Cre:Cre-; Pink1:flox/flox	13.06.2015	30.09.2015	3.63	02.11.2015	13.01.2016		03.12.2015
680	30157213	m	Cre:Cre-; Pink1:flox/flox	13.06.2015	30.09.2015	3.63	02.11.2015	15.01.2016	20.01.2016	14.12.2015
710	30157360	m	Cre:Cre-; Pink1:flox/flox	13.06.2015	30.09.2015	3.63	02.11.2015	15.01.2016		14.12.2015
719	30157369	m	Cre:Cre-; Pink1:flox/flox	13.06.2015	30.09.2015	3.63		12.01.2016	20.01.2016	23.12.2016
790	30157440	m	Cre:Cre-; Pink1:flox/flox	15.06.2015	30.09.2015	3.57	02.11.2015	13.01.2016	20.01.2016	10.12.2015
814	30158282	m	Cre:Cre-; Pink1:flox/flox	19.06.2015	02.11.2015	4.53		19.01.2016	27.01.2016	15.12.2015
817	30158285	m	Cre:Cre-; Pink1:flox/flox	19.06.2015	02.11.2015	4.53		18.01.2016	25.01.2016	15.12.2015
823	30158291	m	Cre:Cre-; Pink1:flox/flox	19.06.2015	02.11.2015	4.53	02.11.2015	19.01.2016	27.01.2016	17.12.2015
903	30159751	m	Cre:Cre-; Pink1:flox/flox	05.07.2015	02.11.2015	4.00	02.11.2015		28.01.2016	
929	30160674	m	Cre:Cre-; Pink1:flox/flox	10.07.2015	02.11.2015	3.83	02.11.2015		29.01.2016	
653	30157186	f	Cre:Cre+; Pink1:wt/wt	12.06.2015	30.09.2015	3.67	02.11.2015	13.01.2016	21.01.2016	09.12.2015
688	30157221	f	Cre:Cre+; Pink1:wt/wt	13.06.2015	30.09.2015	3.63	02.11.2015			
690	30157223	f	Cre:Cre+; Pink1:wt/wt	13.06.2015	30.09.2015	3.63	02.11.2015			
703	30157235	f	Cre:Cre+; Pink1:wt/wt	13.06.2015	30.09.2015	3.63	02.11.2015	15.01.2016		14.12.2015
707	30157239	f	Cre:Cre+; Pink1:wt/wt	14.06.2015	02.10.2015	3.67		19.01.2016	25.01.2016	15.12.2015
714	30157364	f	Cre:Cre+; Pink1:wt/wt	13.06.2015	30.09.2015	3.63	02.11.2015	12.01.2016	20.01.2016	10.12.2015
728	30157378	f	Cre:Cre+; Pink1:wt/wt	13.06.2015	02.10.2015	3.70	02.11.2015	13.01.2016	21.01.2016	09.12.2015
747	30157397	f	Cre:Cre+; Pink1:wt/wt	14.06.2015	02.10.2015	3.67	02.11.2015	15.01.2016	27.01.2016	16.12.2015
763	30157413	f	Cre:Cre+; Pink1:wt/wt	14.06.2015	02.10.2015	3.67	02.11.2015	18.01.2016	28.01.2016	15.12.2015
771	30157421	f	Cre:Cre+; Pink1:wt/wt	15.06.2015	02.10.2015	3.63		19.01.2016	29.01.2016	15.12.2015
931	30160676	f	Cre:Cre+; Pink1:wt/wt	10.07.2015	02.11.2015	3.83			28.01.2016	
683	30157216	f	Cre:Cre+; Pink1:flox/flox	13.06.2015	30.09.2015	3.63	02.11.2015	14.01.2016	21.01.2016	
687	30157220	f	Cre:Cre+; Pink1:flox/flox	13.06.2015	30.09.2015	3.63	02.11.2015			
726	30157376	f	Cre:Cre+; Pink1:flox/flox	13.06.2015	30.09.2015	3.63		14.01.2016	27.01.2016	14.12.2015
727	30157377	f	Cre:Cre+; Pink1:flox/flox	13.06.2015	02.10.2015	3.70	02.11.2015	12.01.2016	20.01.2016	10.12.2015
734	30157384	f	Cre:Cre+; Pink1:flox/flox	13.06.2015	02.10.2015	3.70	02.11.2015	13.01.2016	21.01.2016	09.12.2015
735	30157385	f	Cre:Cre+; Pink1:flox/flox	13.06.2015	02.10.2015	3.70	02.11.2015	15.01.2016	27.01.2016	16.12.2015
756	30157406	f	Cre:Cre+; Pink1:flox/flox	14.06.2015	02.10.2015	3.67			29.01.2016	
773	30157423	f	Cre:Cre+; Pink1:flox/flox	15.06.2015	02.10.2015	3.63	02.11.2015	19.01.2016	25.01.2016	16.12.2015
981	30161928	f	Cre:Cre+; Pink1:flox/flox	25.07.2015	19.11.2015	3.90		18.01.2016	28.01.2016	15.12.2015
985	30161932	f	Cre:Cre+; Pink1:flox/flox	25.07.2015	19.11.2015	3.90		19.01.2016		15.12.2015
993	30161950	f	Cre:Cre+; Pink1:flox/flox	26.07.2015	19.11.2015	3.87				17.12.2015
656	30157189	f	Cre:Cre-; Pink1:flox/flox	12.06.2015	30.09.2015	3.67	02.11.2015	13.01.2016	20.01.2016	03.12.2015
659	30157192	f	Cre:Cre-; Pink1:flox/flox	12.06.2015	30.09.2015	3.67		18.01.2016		23.12.2015
664	30157197	f	Cre:Cre-; Pink1:flox/flox	12.06.2015	30.09.2015	3.67		14.01.2016	25.01.2016	14.12.2015
672	30157205	f	Cre:Cre-; Pink1:flox/flox	13.06.2015	30.09.2015	3.63		12.01.2016	21.01.2016	09.12.2015
675	30157208	f	Cre:Cre-; Pink1:flox/flox	13.06.2015	30.09.2015	3.63		13.01.2016	21.01.2016	
694	30157227	f	Cre:Cre-; Pink1:flox/flox	13.06.2015	02.10.2015	3.70	02.11.2015			
706	30157238	f	Cre:Cre-; Pink1:flox/flox	14.06.2015	02.10.2015	3.67	02.11.2015	19.01.2016	25.01.2016	15.12.2015
772	30157422	f	Cre:Cre-; Pink1:flox/flox	15.06.2015	02.10.2015	3.63	02.11.2015	15.01.2016	28.01.2016	16.12.2015
783	30157433	f	Cre:Cre-; Pink1:flox/flox	15.06.2015	02.10.2015	3.63	02.11.2015	19.01.2016	28.01.2016	16.12.2015
799	30157449	f	Cre:Cre-; Pink1:flox/flox	15.06.2015	02.10.2015	3.63	02.11.2015		29.01.2016	17.12.2015

TH = immunohistochemical staining of TH^+ cells, 5-HT = immunohistochemical staining of $5\text{-}HT^+$ cells, HPLC = quantification of neurotransmitter content via HPLC, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, date est. = estimated date

Table 10: Overview of analysed Pink1 x DAT mice of the cohort 2

Pink1 x DAT, cohort 2

young

							TH	HPLC		
ear nr.	ID C-Streifen	sex	genotype	born	prep	age	date est.	date OB	date STR	date VM
799	30159083	m	Cre:Cre+; Pink1:wt/wt	29.06.2015	20.11.2015	4.80		19.02.2016	11.03.2016	10.02.2016
819	30159160	m	Cre:Cre+; Pink1:wt/wt	29.06.2015	20.11.2015	4.80	11.04.2016	25.02.2016	11.03.2016	09.02.2016
835	30159176	m	Cre:Cre+; Pink1:wt/wt	29.06.2015	20.11.2015	4.80	11.04.2016	24.02.2016	11.03.2016	
860	30159217	m	Cre:Cre+; Pink1:wt/wt	29.06.2015	02.11.2015	4.20	11.04.2016	12.02.2016	26.02.2016	03.02.2016
899	30159256	m	Cre:Cre+; Pink1:wt/wt	30.06.2015	02.11.2015	4.17	11.04.2016	12.02.2016	26.02.2016	02.02.2016
909	30159598	m	Cre:Cre+; Pink1:wt/wt	30.06.2015	02.11.2015	4.17		15.02.2016	02.03.2016	02.02.2016
916	30159622	m	Cre:Cre+; Pink1:wt/wt	30.06.2015	03.11.2015	4.20	11.04.2016	19.02.2016	01.03.2016	25.02.2016
932	30159686	m	Cre:Cre+; Pink1:wt/wt	30.06.2015	03.11.2015	4.20	11.04.2016			05.02.2016
954	30159713	m	Cre:Cre+; Pink1:wt/wt	03.07.2015	03.11.2015	4.10	11.04.2016	18.02.2016	02.03.2016	05.02.2016
818	30159159	m	Cre:Cre+; Pink1:flox/flox	29.06.2015	20.11.2015	4.80		24.02.2016	11.03.2016	09.02.2016
820	30159161	m	Cre:Cre+; Pink1:flox/flox	29.06.2015	20.11.2015	4.80				09.02.2016
825	30159166	m	Cre:Cre+; Pink1:flox/flox	29.06.2015	20.11.2015	4.80	11.04.2016	24.02.2016	11.03.2016	
833	30159174	m	Cre:Cre+; Pink1:flox/flox	29.06.2015	02.11.2015	4.20		15.02.2016	10.03.2016	03.02.2016
872	30159229	m	Cre:Cre+; Pink1:flox/flox	29.06.2015	02.11.2015	4.20	11.04.2016			01.02.2016
874	30159231	m	Cre:Cre+; Pink1:flox/flox	29.06.2015	02.11.2015	4.20		12.02.2016	26.02.2016	03.02.2016
892	30159249	m	Cre:Cre+; Pink1:flox/flox	30.06.2015	03.11.2015	4.20	11.04.2016	12.02.2016	01.03.2016	05.02.2016
897	30159254	m	Cre:Cre+; Pink1:flox/flox	30.06.2015	03.11.2015	4.20	11.04.2016	18.02.2016	01.03.2016	04.02.2016
938	30159692	m	Cre:Cre+; Pink1:flox/flox	30.06.2015	03.11.2015	4.20	11.04.2016	19.02.2016	01.03.2016	25.02.2016
949	30159708	m	Cre:Cre+; Pink1:flox/flox	03.07.2015	03.11.2015	4.10	11.04.2016	18.02.2016	09.03.2016	
743	30159018	m	Cre:Cre-; Pink1:flox/flox	27.06.2015	19.11.2015	4.83	11.04.2016	15.02.2016	01.03.2016	01.02.2016
780	30159055	m	Cre:Cre-; Pink1:flox/flox	29.06.2015	19.11.2015	4.77		19.02.2016	10.03.2016	04.02.2016
786	30159070	m	Cre:Cre-; Pink1:flox/flox	29.06.2015	19.11.2015	4.77		19.02.2016	26.02.2016	05.02.2016
842	30159183	m	Cre:Cre-; Pink1:flox/flox	29.06.2015	19.11.2015	4.77	11.04.2016	19.02.2015	11.03.2016	10.02.2016
867	30159224	m	Cre:Cre-; Pink1:flox/flox	30.06.2015	02.11.2015	4.17	11.04.2016	15.02.2015	25.02.2106	03.02.2016
876	30159233	m	Cre:Cre-; Pink1:flox/flox	29.06.2015	02.11.2015	4.20	11.04.2016		02.03.2016	
908	30159597	m	Cre:Cre-; Pink1:flox/flox	30.06.2015	02.11.2015	4.17	11.04.2016		10.03.2016	
951	30159710	m	Cre:Cre-; Pink1:flox/flox	03.07.2015	02.11.2015	4.07	11.04.2016	12.02.2015	02.03.2016	03.02.2016
992	30161146	m	Cre:Cre-; Pink1:flox/flox	20.07.2015	19.11.2015	4.07	11.04.2016	24.02.2015		10.02.2016
1027	30161729	m	Cre:Cre-; Pink1:flox/flox	24.07.2015	19.11.2015	3.93	11.04.2016	25.02.2015		09.02.2016
744	30159019	f	Cre:Cre+; Pink1:wt/wt	27.06.2015	19.11.2015	4.83	11.04.2016			09.02.2016
747	30159022	f	Cre:Cre+; Pink1:wt/wt	27.06.2015	19.11.2015	4.83	11.04.2016	24.02.2016	09.03.2016	10.02.2016
816	30159157	f	Cre:Cre+; Pink1:wt/wt	29.06.2015	19.11.2015	4.77		24.02.2016	11.03.2016	
883	30159240	f	Cre:Cre+; Pink1:wt/wt	30.06.2015	19.11.2015	4.73	11.04.2016			
894	30159251	f	Cre:Cre+; Pink1:wt/wt	30.06.2015	02.11.2015	4.17	11.04.2016	15.02.2016	26.02.2016	03.02.2016
895	30159252	f	Cre:Cre+; Pink1:wt/wt	30.06.2015	02.11.2015	4.17	11.04.2016	12.02.2016	26.02.2016	01.02.2016
910	30159599	f	Cre:Cre+; Pink1:wt/wt	30.06.2015	02.11.2015	4.17	11.04.2016	12.02.2016	01.03.2016	03.02.2016
931	30159650	f	Cre:Cre+; Pink1:wt/wt	30.06.2015	02.11.2015	4.17	11.04.2016	18.02.2016	01.03.2016	05.02.2016
965	30159723	f	Cre:Cre+; Pink1:wt/wt	05.07.2015	03.11.2015	4.03	11.04.2016	18.02.2016	02.03.2016	05.02.2016
973	30159731	f	Cre:Cre+; Pink1:wt/wt	07.07.2015	03.11.2015	3.97		18.02.2016	09.03.2016	10.02.2016
736	30158914	f	Cre:Cre+; Pink1:flox/flox	27.06.2015	19.11.2015	4.83	11.04.2016	18.02.2015	02.03.2016	04.02.2016
753	30159028	f	Cre:Cre+; Pink1:flox/flox	28.06.2015	19.11.2015	4.80	11.04.2016	18.02.2015	02.03.2016	04.02.2016
777	30159052	f	Cre:Cre+; Pink1:flox/flox	28.06.2015	19.11.2015	4.80	11.04.2016			25.02.2016
781	30159056	f	Cre:Cre+; Pink1:flox/flox	29.06.2015	19.11.2015	4.77	11.04.2016	25.02.2015	10.03.2016	
889	30159246	f	Cre:Cre+; Pink1:flox/flox	30.06.2015	02.11.2015	4.17		12.02.2015	10.03.2016	
913	30159602	f	Cre:Cre+; Pink1:flox/flox	30.06.2015	02.11.2015	4.17	11.04.2016	12.02.2015	26.02.2016	02.02.2016
925	30159644	f	Cre:Cre+; Pink1:flox/flox	30.06.2015	02.11.2015	4.17				01.02.2016
940	30159694	f	Cre:Cre+; Pink1:flox/flox	30.06.2015	02.11.2015	4.17	11.04.2016	15.02.2015	25.02.2016	02.02.2016
947	30159706	f	Cre:Cre+; Pink1:flox/flox	30.06.2015	02.11.2015	4.17	11.04.2016	19.02.2015	01.03.2016	01.02.2016
983	30161137	f	Cre:Cre+; Pink1:flox/flox	20.07.2015	19.11.2015	4.07		24.02.2015	09.03.2016	10.02.2016
761	30159036	f	Cre:Cre-; Pink1:flox/flox	28.06.2015	19.11.2015	4.80	11.04.2016			
775	30159050	f	Cre:Cre-; Pink1:flox/flox	28.06.2015	19.11.2015	4.80	11.04.2016			
864	30159221	f	Cre:Cre-; Pink1:flox/flox	29.06.2015	02.11.2015	4.20		15.02.2015	26.02.2016	02.02.2016
888	30159245	f	Cre:Cre-; Pink1:flox/flox	30.06.2015	02.11.2015	4.17		15.02.2015	25.02.2016	
896	30159253	f	Cre:Cre-; Pink1:flox/flox	30.06.2015	02.11.2015	4.17	11.04.2016	15.02.2015	26.02.2016	01.02.2016
927	30159646	f	Cre:Cre-; Pink1:flox/flox	30.06.2015	02.11.2015	4.17	11.04.2016	19.02.2015	02.03.2016	03.02.2016
930	30159649	f	Cre:Cre-; Pink1:flox/flox	30.06.2015	03.11.2015	4.20	11.04.2016	18.02.2015	02.03.2016	02.02.2016
961	30159719	f	Cre:Cre-; Pink1:flox/flox	04.07.2015	03.11.2015	4.07	11.04.2016	18.02.2015	02.03.2016	04.02.2016
962	30159720	f	Cre:Cre-; Pink1:flox/flox	04.07.2015	03.11.2015	4.07	11.04.2016	24.02.2015	11.03.2016	04.02.2016
966	30159724	f	Cre:Cre-; Pink1:flox/flox	05.07.2015	03.11.2015	4.03	11.04.2016	24.02.2015	09.03.2016	10.02.2016

TH = immunohistochemical staining of TH^+ cells, 5-HT = immunohistochemical staining of $5\text{-}HT^+$ cells, HPLC = quantification of neurotransmitter content via HPLC, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, date est. = estimated date

Table A11: ANOVA table of TH⁺ cells and 5-HT⁺ cells of young and mid-aged Pink1 x Pet and Pink1 x DAT mice

Pink1 x Pet and Pink1 x DAT, young and mid-aged, TH⁺ cells and 5-HT⁺ cells																				
		Genotype		Sex		Interaction		post-hoc Bonferroni p-value			males		post-hoc Bonferroni p-value			females		post-hoc Bonferroni p-value		
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	Genotype F-value	Genotype p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	Genotype F-value	Genotype p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
TH	Pink1 x Pet, young	1.170	0.322	0.271	0.606	0.574	0.569	1.000	0.357	1.000	3.597	0.049	0.484	0.046	0.716	0.089	0.916	1.000	1.000	1.000
	Pink1 x Pet, mid-aged	2.123	0.134	1.019	0.320	0.290	0.750	1.000	0.416	0.171	1.070	0.364	1.000	1.000	0.484	1.398	0.273	1.000	0.416	0.611
	Pink1 x DAT, young	0.391	0.679	1.306	0.260	1.609	0.213	1.000	1.000	1.000	0.648	0.535	1.000	0.892	1.000	1.325	0.288	1.000	0.497	0.565
	Pink1 x DAT, mid-aged	0.646	0.529	0.324	0.573	1.016	0.371	0.769	1.000	1.000	1.522	0.241	0.349	1.000	0.583	0.044	0.957	1.000	1.000	1.000
5-HT	Pink1 x Pet, mid-aged	0.147	0.721	34.778	0.004	0.004	0.953				0.158	0.730				0.038	0.864			
	Pink1 x DAT, mid-aged	0.005	0.945	0.011	0.921	0.910	0.368				0.359	0.582				0.727	0.442			

Table A12: Summary of results of TH⁺ cells and 5-HT⁺ fibres of young and mid-aged Pink1 x Pet and Pink1 x DAT mice

TH⁺ cells

Pink1 x Pet	young										mid-aged									
	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
	males	ctrl. 1	7	5897.86	459.08	female	ctrl. 1	8	5917.50	1910.70	males	ctrl. 1	7	7431.43	1584.49	females	ctrl. 1	7	7355.00	580.25
		mt	7	5074.29	1328.32		mt	6	6042.50	1907.09		mt	7	8058.57	1812.43		mt	7	7200.00	1932.46
		ctrl. 2	7	6585.00	1168.27		ctrl. 2	6	6342.50	1842.34		ctrl. 2	7	8593.57	920.17		ctrl. 2	7	8241.43	818.79

Pink1 x DAT	young										mid-aged									
	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
	males	ctrl. 1	7	7688.57	1463.94	female	ctrl. 1	8	7173.75	1167.46	males	ctrl. 1	8	8898.75	1490.36	females	ctrl. 1	8	8301.25	984.08
		mt	6	8300.00	1017.11		mt	7	7092.86	1648.90		mt	8	7680.00	1446.86		mt	7	8387.86	1219.64
		ctrl. 2	8	7601.25	1077.97		ctrl. 2	8	8036.25	943.58		ctrl. 2	8	7901.25	1527.35		ctrl. 2	7	8509.29	1778.16

5-HT⁺ cells

Pink1 x Pet	mid-aged									
	age	groups	n	mean	SD	age	groups	n	mean	SD
	males	mt	2	6327.16	429.04	females	mt	2	10290.11	858.58
		ctrl.2	2	6024.26	989.18		ctrl.2	2	10071.52	1337.99

Pink1 x DAT	mid-aged									
	age	groups	n	mean	SD	age	groups	n	mean	SD
	males	mt	3	10468.32	2019.66	females	mt	3	9557.75	363.92
		ctrl.2	3	9584.95	1564.52		ctrl.2	3	10318.70	1502.51

Table A13: ANOVA table of HPLC measurements of young Pink1 x Pet mice

		Pink1 x Pet, young, HPLC									males					females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value				Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2		F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
OB	DOPAC	0.491	0.615	1.245	0.271	0.187	0.830	1.000	1.000	1.000	0.041 0.960	1.000	1.000	1.000		1.088	0.355	1.000	0.585	0.724
	NE	0.712	0.496	1.510	0.226	2.337	0.109	1.000	1.000	0.823	1.931 0.170	1.000	0.207	0.591		0.757	0.481	0.864	0.932	1.000
	5-HIAA	1.871	0.167	2.600	0.114	2.802	0.072	1.000	0.640	0.193	2.879 0.079	1.000	0.128	0.180		1.066	0.362	0.508	0.997	1.000
	HVA	0.577	0.566	0.296	0.589	0.687	0.509	0.882	1.000	1.000	0.533 0.595	1.000	1.000	0.945		0.776	0.473	1.000	0.775	1.000
	DA	1.148	0.327	3.142	0.084	0.205	0.815	0.412	1.000	1.000	0.339 0.716	1.000	1.000	1.000		0.974	0.394	0.571	1.000	1.000
	3-MT	1.662	0.202	0.038	0.846	1.257	0.295	0.275	1.000	0.533	2.220 0.133	0.170	1.000	0.419		0.026	0.974	1.000	1.000	1.000
	5-HT	1.628	0.208	0.121	0.730	1.569	0.220	1.000	0.643	0.263	2.711 0.090	1.000	0.194	0.151		0.175	0.841	1.000	1.000	1.000
STR	DOPAC	0.662	0.521	1.646	0.206	0.620	0.543	1.000	0.939	1.000	0.292 0.750	1.000	1.000	1.000		0.918	0.415	1.000	0.570	1.000
	NE	0.748	0.480	0.503	0.482	3.971	0.026	1.000	1.000	0.750	3.517 0.048	0.046	0.867	0.408		1.260	0.304	0.611	0.503	1.000
	5-HIAA	0.336	0.717	7.272	0.010	2.225	0.121	1.000	1.000	1.000	0.695 0.510	0.860	1.000	1.000		1.984	0.163	0.201	0.541	1.000
	HVA	0.398	0.674	0.614	0.438	0.554	0.579	1.000	1.000	1.000	0.255 0.777	1.000	1.000	1.000		0.850	0.442	1.000	0.662	1.000
	DA	0.175	0.840	0.735	0.396	0.539	0.587	1.000	1.000	1.000	0.291 0.750	1.000	1.000	1.000		0.429	0.657	1.000	1.000	1.000
	3-MT	1.066	0.354	1.210	0.278	0.018	0.982	1.000	0.461	1.000	0.471 0.631	1.000	1.000	1.000		0.661	0.527	1.000	0.790	1.000
	5-HT	0.409	0.667	0.264	0.610	1.631	0.208	1.000	1.000	1.000	2.656 0.094	0.110	0.367	1.000		0.190	0.828	1.000	1.000	1.000
VM	DOPAC	0.051	0.951	0.379	0.542	0.456	0.637	1.000	1.000	1.000	0.169 0.846	1.000	1.000	1.000		0.292	0.750	1.000	1.000	1.000
	NE	0.158	0.854	0.052	0.821	0.382	0.685	1.000	1.000	1.000	0.084 0.920	1.000	1.000	1.000		0.379	0.689	1.000	1.000	1.000
	5-HIAA	1.551	0.224	8.955	0.005	0.475	0.625	1.000	0.666	0.288	2.007 0.159	1.000	0.398	0.228		0.183	0.834	1.000	1.000	1.000
	HVA	1.215	0.307	0.536	0.468	0.317	0.730	0.475	1.000	0.662	0.843 0.445	1.000	1.000	0.633		0.742	0.488	0.715	1.000	1.000
	DA	0.800	0.456	0.462	0.500	1.474	0.241	0.806	0.874	1.000	0.235 0.792	1.000	1.000	1.000		1.260	0.304	0.488	0.634	1.000
	3-MT	1.121	0.336	0.496	0.485	0.236	0.791	1.000	.695	.601	0.655 0.530	1.000	.993	.977		0.592	0.562	1.000	1.000	.949
	5-HT	1.327	0.276	0.256	0.615	0.906	0.412	1.000	1.000	0.333	1.957 0.166	1.000	0.299	0.310		0.636	0.539	0.846	1.000	1.000

DOPAC = 3,4-Dihydroxyphenylacetic acid, NE = Norepinephrine, 5-HIAA = 5-Hydroxyindole-3-acetic acid, HVA = Homovanillic acid, DA = Dopamine, 3-MT = 3-Methoxytyramine, 5-HT = Serotonin, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A14: ANOVA table of HPLC measurements of mid-aged Pink1 x Pet mice

		Pink1 x Pet, mid-aged, HPLC									males				females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
OB	DOPAC	0.281	0.757	2.387	0.130	0.325	0.724	1.000	1.000	1.000	0.002 0.998	1.000	1.000	1.000	0.416	0.665	1.000	1.000	1.000
	NE	0.316	0.731	1.166	0.286	0.575	0.567	1.000	1.000	1.000	0.048 0.953	1.000	1.000	1.000	0.543	0.589	1.000	1.000	1.000
	5-HIAA	0.903	0.413	17.130	0.000	0.075	0.928	1.000	0.855	0.675	1.886 0.176	1.000	0.245	0.462	0.312	0.735	1.000	1.000	1.000
	HVA	0.172	0.843	2.588	0.115	0.252	0.779	1.000	1.000	1.000	0.008 0.992	1.000	1.000	1.000	0.282	0.757	1.000	1.000	1.000
	DA	0.039	0.961	1.018	0.319	0.199	0.820	1.000	1.000	1.000	0.070 0.932	1.000	1.000	1.000	0.137	0.873	1.000	1.000	1.000
	3-MT																		
	5-HT	0.479	0.623	3.142	0.084	0.038	0.962	1.000	1.000	1.000	0.293 0.749	1.000	1.000	1.000	0.245	0.785	1.000	1.000	1.000
STR	DOPAC	5.007	0.011	0.496	0.485	0.681	0.512	1.000	0.049	0.016	3.240 0.059	1.000	0.224	0.073	1.874	0.178	1.000	0.305	0.348
	NE	2.822	0.071	0.077	0.782	0.583	0.563	0.990	0.525	0.068	1.943 0.168	1.000	1.000	0.186	0.970	0.395	1.000	0.914	0.607
	5-HIAA	4.429	0.018	4.851	0.033	0.272	0.763	1.000	0.132	0.018	2.371 0.118	1.000	0.276	0.180	2.314	0.124	0.926	0.841	0.130
	HVA	5.120	0.010	0.480	0.492	0.227	0.798	0.966	0.117	0.009	2.411 0.114	1.000	0.560	0.124	3.528	0.048	1.000	0.200	0.057
	DA	4.933	0.012	0.054	0.818	0.550	0.581	1.000	0.068	0.015	2.208 0.135	1.000	0.670	0.148	5.813	0.010	1.000	0.015	0.039
	3-MT	5.578	0.007	0.105	0.748	0.902	0.414	1.000	0.043	0.009	3.343 0.055	1.000	0.332	0.056	2.915	0.076	1.000	0.105	0.224
	5-HT	3.432	0.042	0.091	0.765	0.945	0.397	1.000	0.143	0.057	2.374 0.118	1.000	0.348	0.155	1.232	0.312	1.000	0.583	0.549
VM	DOPAC	2.586	0.087	1.790	0.188	0.731	0.487	0.968	0.635	0.085	3.099 0.066	1.000	0.122	0.135	1.096	0.353	0.719	1.000	0.581
	NE	0.434	0.651	1.868	0.179	0.392	0.678	1.000	1.000	1.000	0.711 0.503	1.000	0.810	1.000	0.227	0.799	1.000	1.000	1.000
	5-HIAA	1.572	0.220	26.694	0.000	0.498	0.612	0.824	1.000	0.261	1.015 0.379	1.000	0.758	0.639	1.042	0.370	0.697	1.000	0.654
	HVA	0.478	0.624	2.241	0.142	0.808	0.453	1.000	1.000	1.000	0.345 0.712	1.000	1.000	1.000	0.740	0.489	0.713	1.000	1.000
	DA	0.163	0.850	0.306	0.583	0.982	0.383	1.000	1.000	1.000	0.380 0.688	1.000	1.000	1.000	0.701	0.507	1.000	0.750	1.000
	3-MT	1.809	0.176	1.180	0.283	0.458	0.636	0.222	1.000	0.544	0.352 0.708	1.000	1.000	1.000	1.762	0.196	0.230	1.000	0.811
	5-HT	0.092	0.912	1.108	0.298	0.135	0.874	1.000	1.000	1.000	0.345 0.712	1.000	1.000	1.000	0.003	0.997	1.000	1.000	1.000

DOPAC = 3,4-Dihydroxyphenylacetic acid, NE = Norepinephrine, 5-HIAA = 5-Hydroxyindole-3-acetic acid, HVA = Homovanillic acid, DA = Dopamine, 3-MT = 3-Methoxytyramine, 5-HT = Serotonin, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, black = data could not be measured, red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A15: ANOVA table of HPLC measurements of young Pink1 x DAT mice

		Pink1 x Dat, young, HPLC									males					females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value				Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2		F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
OB	DOPAC	2.477	0.096	2.930	0.094	0.719	0.493	0.524	1.000	0.100	3.219 0.060	0.367	1.000	0.062		0.243	0.786	1.000	1.000	1.000
	NE	0.072	0.931	0.791	0.379	6.092	0.005	1.000	1.000	1.000	3.427 0.052	0.526	0.716	0.048		2.879	0.079	0.955	0.556	0.079
	5-HIAA	0.349	0.707	5.838	0.020	1.193	0.313	1.000	1.000	1.000	1.124 0.344	0.653	0.599	1.000		0.220	0.805	1.000	1.000	1.000
	HVA	5.212	0.010	0.166	0.686	1.607	0.213	0.166	0.676	0.008	5.370 0.013	0.171	0.694	0.012		0.624	0.545	1.000	1.000	0.838
	DA	1.104	0.341	0.108	0.744	2.991	0.061	0.573	1.000	0.665	5.108 0.016	0.245	0.569	0.013		0.572	0.573	1.000	0.917	1.000
	3-MT	0.704	0.501	0.375	0.543	1.133	0.332	1.000	1.000	0.810	2.692 0.091	0.102	1.000	0.403		0.240	0.789	1.000	1.000	1.000
	5-HT	0.150	0.861	1.693	0.200	1.059	0.356	1.000	1.000	1.000	0.564 0.578	0.904	1.000	1.000		0.633	0.541	1.000	1.000	0.883
STR	DOPAC	0.211	0.811	1.738	0.195	1.329	0.276	1.000	1.000	1.000	1.555 0.235	1.000	1.000	0.277		0.664	0.525	1.000	0.963	1.000
	NE	0.101	0.904	1.176	0.284	0.853	0.433	1.000	1.000	1.000	0.412 0.668	1.000	1.000	1.000		1.368	0.276	0.599	0.430	1.000
	5-HIAA	0.009	0.991	3.152	0.083	0.476	0.625	1.000	1.000	1.000	0.598 0.559	1.000	1.000	0.948		0.129	0.880	1.000	1.000	1.000
	HVA	0.389	0.680	0.520	0.475	1.029	0.366	1.000	1.000	1.000	1.001 0.385	1.000	1.000	0.523		0.680	0.518	1.000	0.845	1.000
	DA	0.947	0.396	2.442	0.126	0.724	0.491	0.820	0.645	1.000	2.836 0.081	0.093	1.000	0.340		0.629	0.543	1.000	0.838	1.000
	3-MT	0.819	0.448	2.336	0.134	2.059	0.140	0.631	1.000	1.000	3.144 0.064	0.380	1.000	0.066		0.922	0.413	1.000	0.571	1.000
	5-HT	0.948	0.396	2.665	0.110	0.471	0.628	0.684	0.760	1.000	1.386 0.272	0.368	1.000	0.756		0.586	0.565	1.000	0.883	1.000
VM	DOPAC	0.453	0.639	3.526	0.068	0.580	0.564	1.000	1.000	1.000	0.167 0.847	1.000	1.000	1.000		0.949	0.404	0.595	1.000	1.000
	NE	0.274	0.762	0.425	0.518	1.610	0.212	1.000	1.000	1.000	1.492 0.248	1.000	0.798	0.315		0.369	0.696	1.000	1.000	1.000
	5-HIAA	1.047	0.360	6.257	0.016	2.089	0.137	0.886	1.000	0.525	1.604 0.225	1.000	0.405	0.410		1.639	0.219	0.308	0.581	1.000
	HVA	0.959	0.392	1.081	0.304	0.712	0.497	1.000	0.506	1.000	0.227 0.799	1.000	1.000	1.000		1.654	0.216	0.447	0.360	1.000
	DA	1.343	0.272	4.940	0.032	2.271	0.116	0.505	0.466	1.000	0.073 0.930	1.000	1.000	1.000		3.003	0.072	0.142	0.144	1.000
	3-MT	0.077	0.926	0.048	0.827	0.413	0.664	1.000	1.000	1.000	0.280 0.758	1.000	1.000	1.000		0.190	0.828	1.000	1.000	1.000
	5-HT	0.122	0.886	1.693	0.200	0.726	0.490	1.000	1.000	1.000	0.544 0.589	1.000	1.000	1.000		0.364	0.699	1.000	1.000	1.000

DOPAC = 3,4-Dihydroxyphenylacetic acid, NE = Norepinephrine, 5-HIAA = 5-Hydroxyindole-3-acetic acid, HVA = Homovanillic acid, DA = Dopamine, 3-MT = 3-Methoxytyramine, 5-HT = Serotonin, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A16: ANOVA table of HPLC measurements of mid-aged Pink1 x DAT mice

		Pink1 x Dat, mid-aged, HPLC									males				females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
OB	DOPAC	2.344	0.108	0.818	0.371	0.013	0.987	1.000	0.185	0.224	1.467 0.253	1.000	0.477	0.441	1.006	0.383	1.000	0.627	0.793
	NE	1.856	0.169	0.231	0.634	1.076	0.350	1.000	0.833	0.185	0.384 0.686	1.000	1.000	1.000	3.207	0.061	0.269	1.000	0.069
	5-HIAA	0.036	0.965	11.119	0.002	1.021	0.369	1.000	1.000	1.000	0.611 0.552	1.000	1.000	0.856	0.496	0.616	1.000	1.000	0.994
	HVA	1.456	0.245	3.503	0.068	1.485	0.238	0.293	1.000	0.941	0.484 0.623	1.000	1.000	1.000	1.584	0.229	0.313	1.000	0.613
	DA	0.929	0.403	0.154	0.697	1.589	0.216	1.000	0.610	0.937	1.221 0.315	0.432	0.875	1.000	1.280	0.299	1.000	1.000	0.374
	3-MT	0.126	0.882	3.218	0.080	0.149	0.862	1.000	1.000	1.000	0.208 0.814	1.000	1.000	1.000	0.087	0.917	1.000	1.000	1.000
	5-HT	0.014	0.986	6.142	0.017	2.037	0.143	1.000	1.000	1.000	1.306 0.292	0.472	0.602	1.000	0.849	0.442	0.669	1.000	1.000
STR	DOPAC	1.428	0.251	9.231	0.004	0.292	0.748	1.000	0.650	0.346	2.243 0.131	1.000	0.161	0.443	0.669	0.523	1.000	1.000	0.781
	NE	1.232	0.302	9.721	0.003	0.883	0.421	1.000	0.584	0.509	0.049 0.952	1.000	1.000	1.000	1.309	0.291	1.000	0.605	0.467
	5-HIAA	0.104	0.901	18.120	0.000	0.078	0.925	1.000	1.000	1.000	0.326 0.725	1.000	1.000	1.000	0.001	0.999	1.000	1.000	1.000
	HVA	4.155	0.023	7.468	0.009	2.098	0.135	1.000	0.024	0.142	0.753 0.483	1.000	0.820	1.000	4.009	0.034	1.000	0.053	0.093
	DA	4.216	0.021	7.011	0.011	1.296	0.284	1.000	0.029	0.087	1.678 0.211	1.000	0.487	0.314	2.941	0.075	1.000	0.089	0.288
	3-MT	1.874	0.166	7.186	0.010	0.831	0.443	0.958	0.179	1.000	1.528 0.240	0.331	0.636	1.000	1.302	0.293	1.000	0.441	0.669
	5-HT	1.788	0.180	7.996	0.007	0.967	0.389	1.000	0.219	0.598	1.559 0.234	1.000	1.000	0.276	1.333	0.285	0.944	0.365	1.000
VM	DOPAC	2.415	0.102	0.313	0.579	0.621	0.542	0.292	0.137	1.000	0.924 0.412	0.585	1.000	1.000	2.620	0.096	0.926	0.098	0.683
	NE	3.347	0.045	0.099	0.755	0.118	0.889	0.142	0.063	1.000	1.800 0.190	0.442	0.282	1.000	1.603	0.225	0.525	0.332	1.000
	5-HIAA	1.613	0.211	4.040	0.051	0.203	0.817	0.583	0.281	1.000	1.358 0.279	0.432	0.608	1.000	0.690	0.513	1.000	0.761	1.000
	HVA	2.640	0.083	0.012	0.912	0.211	0.811	0.142	0.180	1.000	1.519 0.242	0.336	0.629	1.000	1.254	0.306	0.738	0.447	1.000
	DA	3.160	0.053	0.245	0.623	1.447	0.247	0.969	0.050	0.425	1.307 0.292	1.000	0.903	0.382	2.711	0.090	0.427	0.098	1.000
	3-MT	0.483	0.624	0.042	0.841	3.285	0.060	.861	1.000	1.000	1.805 0.214	.672	.299	1.000	2.533	0.134			
	5-HT	4.195	0.022	0.017	0.896	0.773	0.468	0.838	0.019	0.250	1.624 0.221	1.000	0.439	0.368	3.465	0.050	0.306	0.051	1.000

DOPAC = 3,4-Dihydroxyphenylacetic acid, NE = Norepinephrine, 5-HIAA = 5-Hydroxyindole-3-acetic acid, HVA = Homovanillic acid, DA = Dopamine, 3-MT = 3-Methoxytyramine, 5-HT = Serotonin, OB = olfactory bulb, STR = striatum, VM = ventral midbrain, black = data could not be measured, red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A17: Summary of results of DA [pg/ mg] content of young and mid-aged Pink1 x Pet and Pink1 x DAT mice

DA in OB

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	462.66	182.76	female	ctrl. 1	8	379.48	82.06	males	ctrl. 1	8	397.19	110.33	female	ctrl. 1	8	430.79	191.95
	mt	8	515.97	107.30		mt	8	479.10	187.12		mt	8	397.66	121.14		mt	8	412.31	26.67
	ctrl. 2	8	506.70	111.53		ctrl. 2	8	407.51	152.58		ctrl. 2	8	380.52	75.64		ctrl. 2	8	457.54	230.53

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	287.11	66.60	female	ctrl. 1	8	352.78	133.84	males	ctrl. 1	8	327.95	49.34	females	ctrl. 1	8	406.75	98.12
	mt	8	361.91	67.22		mt	8	373.31	103.72		mt	8	388.01	72.99		mt	8	364.54	62.00
	ctrl. 2	8	417.37	105.47		ctrl. 2	8	311.45	113.92		ctrl. 2	8	345.22	105.01		ctrl. 2	8	321.69	143.01

DA in STR

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	19,733.95	10,870.98	female	ctrl. 1	8	19,831.30	5,849.62	males	ctrl. 1	8	11,185.51	5,615.27	female	ctrl. 1	8	16,093.62	4,097.84
	mt	8	16,283.67	8,299.20		mt	8	22,237.59	11,727.53		mt	8	16,575.42	5,999.70		mt	8	14,938.40	3,134.77
	ctrl. 2	8	17,706.23	7,804.25		ctrl. 2	8	18,250.55	7,324.78		ctrl. 2	8	24,700.70	20,870.65		ctrl. 2	8	23,420.97	7,794.95

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	17,754.55	5,058.05	female	ctrl. 1	8	18,682.22	9,791.32	males	ctrl. 1	8	4,978.52	765.99	females	ctrl. 1	8	6,572.18	2,323.27
	mt	8	13,372.47	2,584.19		mt	8	16,187.00	3,190.19		mt	8	5,133.20	1,291.07		mt	8	5,681.16	4,364.99
	ctrl. 2	8	14,622.78	3,295.31		ctrl. 2	8	22,739.44	17,651.93		ctrl. 2	8	6,041.35	1,569.30		ctrl. 2	8	9,205.71	1,719.38

DA in VM

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	325.44	101.23	female	ctrl. 1	8	269.98	64.13	males	ctrl. 1	8	129.97	73.98	female	ctrl. 1	8	130.22	56.06
	mt	8	286.29	113.09		mt	8	494.81	523.08		mt	8	105.06	44.73		mt	8	154.23	102.23
	ctrl. 2	8	309.57	128.43		ctrl. 2	8	294.48	109.77		ctrl. 2	8	126.05	62.14		ctrl. 2	8	109.60	59.10

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	350.49	80.32	female	ctrl. 1	8	374.07	79.30	males	ctrl. 1	8	214.34	34.54	females	ctrl. 1	8	257.38	73.90
	mt	8	334.90	116.78		mt	8	491.49	146.52		mt	8	229.70	54.59		mt	8	187.95	92.92
	ctrl. 2	8	350.49	80.32		ctrl. 2	8	324.07	157.01		ctrl. 2	8	260.60	77.59		ctrl. 2	8	292.08	103.86

DA = Dopamine, OB = olfactory bulb, STR = striatum, VM = ventral midbrain

Table A18: Summary of results of 5-HT content [pg/ mg] of young and mid-aged Pink1 x Pet and Pink1 x DAT mice

5-HT in OB

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	535.74	199.62	female	ctrl. 1	8	565.97	148.03	males	ctrl. 1	8	800.21	275.07	female	ctrl. 1	8	983.65	270.19
	mt	8	549.29	197.04		mt	8	621.08	169.28		mt	8	778.16	211.45		mt	8	903.05	253.65
	ctrl. 2	8	755.02	234.47		ctrl. 2	8	592.95	232.08		ctrl. 2	8	863.88	204.26		ctrl. 2	8	1,032.29	528.49

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	352.58	98.75	female	ctrl. 1	8	445.95	164.41	males	ctrl. 1	8	593.78	101.32	females	ctrl. 1	8	810.07	164.28
	mt	8	398.90	92.28		mt	8	402.83	63.16		mt	8	705.98	203.29		mt	8	689.22	142.56
	ctrl. 2	8	378.27	68.33		ctrl. 2	8	389.66	43.06		ctrl. 2	8	604.91	136.50		ctrl. 2	8	778.49	252.58

5-HT in STR

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	673.73	272.30	female	ctrl. 1	8	583.42	199.13	males	ctrl. 1	8	613.43	227.26	female	ctrl. 1	8	878.59	228.63
	mt	8	412.25	213.41		mt	8	662.33	463.16		mt	8	762.28	257.07		mt	8	884.23	131.62
	ctrl. 2	8	600.68	211.69		ctrl. 2	8	567.82	264.54		ctrl. 2	8	1,337.13	1,165.66		ctrl. 2	8	1,091.53	466.22

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	527.41	158.97	female	ctrl. 1	8	593.72	314.30	males	ctrl. 1	8	821.38	133.02	females	ctrl. 1	8	1,096.99	256.65
	mt	8	413.70	96.22		mt	8	486.80	118.06		mt	8	885.37	154.05		mt	8	945.40	333.44
	ctrl. 2	8	444.12	159.58		ctrl. 2	8	665.21	466.22		ctrl. 2	8	949.79	148.51		ctrl. 2	8	1,182.69	287.85

5-HT in VM

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	941.81	192.50	female	ctrl. 1	8	951.72	236.01	males	ctrl. 1	8	613.43	227.26	female	ctrl. 1	8	878.59	228.63
	mt	8	939.57	248.02		mt	8	1,121.65	409.87		mt	8	762.28	257.07		mt	8	884.23	131.62
	ctrl. 2	8	1,139.96	252.49		ctrl. 2	8	1,067.59	246.22		ctrl. 2	8	1,337.13	1,165.66		ctrl. 2	8	1,091.53	466.22

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	8	854.03	259.72	female	ctrl. 1	8	1,008.67	340.92	males	ctrl. 1	8	821.38	133.02	females	ctrl. 1	8	1,096.99	256.65
	mt	8	869.96	296.51		mt	8	1,100.90	287.49		mt	8	885.37	154.05		mt	8	945.40	333.44
	ctrl. 2	8	972.71	166.80		ctrl. 2	8	823.37	541.14		ctrl. 2	8	949.79	148.51		ctrl. 2	8	1,182.69	287.85

Table A19: ANOVA table of OF testing of young Pink1 x Pet mice

		Pink1 x Pet, young, OF									males				females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
whole area	Distance travelled, 5 min [cm]	1.958	0.149	0.241	0.625	0.190	0.828	0.249	1.000	0.272	0.820	0.450	0.649	1.000	1.000	1.422	0.254	0.718	1.000	0.348
	Distance travelled, 10 min [cm]	0.598	0.553	0.125	0.724	1.367	0.262	0.956	1.000	1.000	1.493	0.240	1.000	0.288	0.770	0.623	0.542	1.000	1.000	0.858
	Distance travelled, 15 min [cm]	1.823	0.170	0.271	0.605	0.512	0.601	0.421	0.243	1.000	2.543	0.095	0.711	0.095	0.748	0.465	0.632	1.000	1.000	1.000
	Distance travelled, 20 min [cm]	2.895	0.062	0.133	0.716	0.125	0.883	0.111	0.116	1.000	1.619	0.214	0.499	0.329	1.000	1.476	0.242	0.351	0.506	1.000
	Total distance travelled [cm]	1.561	0.217	0.083	0.774	0.465	0.630	0.257	0.714	1.000	1.420	0.257	0.640	0.376	1.000	0.800	0.457	0.705	1.000	1.000
	Resting time [sec]	6.674	0.002	0.014	0.906	3.985	0.023	0.086	0.001	0.189	2.374	0.110	0.112	0.795	1.000	9.534	0.0005	0.854	0.001	0.008
	Average speed [cm/sec]	0.793	0.457	0.139	0.711	0.894	0.414	0.591	1.000	1.000	0.963	0.393	1.000	0.546	1.000	0.852	0.435	1.000	1.000	0.671
	Number of rearing, 5 min [#]	1.238	0.297	0.016	0.901	1.373	0.260	1.000	1.000	0.262	0.102	0.903	1.000	1.000	1.000	3.025	0.061	0.369	1.000	0.064
	Number of rearing, 10 min [#]	2.364	0.102	0.412	0.523	1.119	0.333	1.000	0.058	0.290	0.955	0.396	0.655	0.838	1.000	2.790	0.075	1.000	0.177	0.131
	Number of rearing, 15 min [#]	1.527	0.225	1.809	0.183	2.113	0.129	1.000	0.284	0.150	0.405	0.671	1.000	1.000	1.000	3.842	0.031	0.655	0.560	0.026
	Number of rearing, 20 min [#]	1.581	0.213	1.595	0.211	1.315	0.275	1.000	0.624	0.115	0.007	0.993	1.000	1.000	1.000	2.802	0.074	0.982	0.686	0.071
	Total number of rearing [#]	2.114	0.129	0.800	0.374	2.102	0.130	1.000	0.194	0.076	0.337	0.716	1.000	1.000	1.000	4.409	0.019	0.736	0.358	0.016
centre	Distance travelled [cm]	1.203	0.307	2.316	0.133	0.003	0.997	0.546	1.000	0.632	0.897	0.418	0.740	1.000	0.852	0.512	0.604	1.000	1.000	1.000
	Resting time [sec]	8.863	0.0004	0.750	0.390	1.957	0.149	0.031	0.0001	0.193	2.997	0.065	0.095	0.194	1.000	8.054	0.001	0.305	0.001	0.060
	Permanance time [sec]	3.487	0.036	1.451	0.233	0.151	0.860	1.000	0.172	0.063	3.068	0.061	1.000	0.114	0.098	1.154	0.327	1.000	0.966	0.445
	Average speed [cm/sec]	1.826	0.169	0.375	0.542	0.420	0.659	1.000	0.188	0.968	1.934	0.162	1.000	0.181	0.561	0.347	0.709	1.000	1.000	1.000
	% Distance travelled, 5 min [%]	0.442	0.645	0.958	0.331	0.185	0.832	1.000	1.000	1.000	0.428	0.656	1.000	1.000	1.000	0.187	0.830	1.000	1.000	1.000
	% Distance travelled, 10 min [%]	2.191	0.120	0.983	0.325	0.588	0.558	1.000	0.408	0.265	2.391	0.108	1.000	0.278	0.134	0.306	0.738	1.000	1.000	1.000
	% Distance travelled, 15 min [%]	1.237	0.297	3.488	0.066	0.348	0.708	1.000	0.820	0.712	1.267	0.296	1.000	0.400	0.697	0.295	0.746	1.000	1.000	1.000
	% Distance travelled, 20 min [%]	1.929	0.153	4.456	0.039	0.278	0.758	0.926	1.000	0.244	2.189	0.129	0.559	1.000	0.153	0.451	0.640	1.000	1.000	1.000
	% Total distance travelled [%]	2.020	0.141	3.776	0.056	0.389	0.679	1.000	0.851	0.265	2.562	0.093	1.000	0.300	0.105	0.314	0.733	1.000	1.000	1.000
	% Time spent in centre, 5 min [%]	1.978	0.146	0.972	0.328	0.319	0.728	1.000	0.904	0.166	0.690	0.509	1.000	0.944	0.886	1.699	0.197	0.754	1.000	0.237
	% Time spent in centre, 10 min [%]	2.516	0.088	0.096	0.758	0.741	0.480	1.000	0.312	0.147	2.729	0.081	1.000	0.215	0.100	0.298	0.744	1.000	1.000	1.000
	% Time spent in centre, 15 min [%]	1.069	0.349	1.245	0.268	0.442	0.645	1.000	0.632	1.000	1.642	0.210	1.000	0.242	1.000	0.218	0.805	1.000	1.000	1.000
	% Time spent in centre, 20 min [%]	3.152	0.049	3.250	0.076	0.134	0.875	1.000	0.208	0.075	2.079	0.142	1.000	0.543	0.155	1.670	0.203	1.000	0.390	0.367
	% Total time spent in centre [%]	3.484	0.036	1.441	0.234	0.148	0.863	1.000	0.173	0.063	3.058	0.061	1.000	0.115	0.099	1.157	0.326	1.000	0.963	0.443
	Latency to enter in the centre [sec]	1.479	0.235	1.014	0.317	1.597	0.210	1.000	0.778	0.628	1.371	0.269	1.000	0.454	0.452	0.080	0.923	1.000	1.000	1.000
	Number of entries in the centre [#]	2.022	0.140	2.709	0.104	0.197	0.821	0.377	1.000	0.238	0.855	0.435	1.000	1.000	0.616	1.313	0.282	0.577	1.000	0.473
periphery	Distance travelled [cm]	2.073	0.134	0.652	0.422	0.967	0.385	0.395	0.268	1.000	2.616	0.089	1.000	0.088	0.499	0.694	0.506	0.740	1.000	1.000
	Resting time [sec]	2.869	0.064	0.155	0.695	3.687	0.030	0.473	0.023	0.472	1.693	0.201	0.247	1.000	0.769	5.491	0.008	1.000	0.018	0.028
	Permanance time [sec]	3.488	0.036	1.451	0.233	0.151	0.860	1.000	0.172	0.063	3.070	0.061	1.000	0.114	0.098	1.154	0.327	1.000	0.966	0.445
	Average speed [cm/sec]	0.911	0.407	0.059	0.809	1.071	0.348	0.601	1.000	0.688	0.493	0.615	1.000	1.000	1.000	1.409	0.257	0.865	1.000	0.328

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A20: ANOVA table of OF testing of mid-aged Pink1 x Pet mice

Pink1 x Pet, mid-aged, OF											males					females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value				Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2		F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
whole area	Distance travelled, 5 min [cm]	0.788	0.459	0.343	0.560	1.760	0.181	1.000	1.000	0.540	0.998 0.382	0.513	1.000	1.000		1.720	0.195	1.000	0.254	0.611
	Distance travelled, 10 min [cm]	0.921	0.404	0.065	0.799	1.025	0.365	1.000	0.408	1.000	0.042 0.959	1.000	1.000	1.000		1.867	0.170	1.000	0.202	0.678
	Distance travelled, 15 min [cm]	0.278	0.758	0.070	0.793	1.197	0.309	1.000	1.000	1.000	1.007 0.378	0.504	1.000	1.000		0.537	0.589	1.000	0.924	1.000
	Distance travelled, 20 min [cm]	0.132	0.876	0.226	0.636	1.193	0.310	1.000	1.000	1.000	0.338 0.716	1.000	1.000	1.000		0.922	0.408	1.000	0.565	1.000
	Total distance travelled [cm]	0.377	0.688	0.012	0.915	1.405	0.253	1.000	1.000	1.000	0.424 0.659	1.000	1.000	1.000		1.400	0.261	1.000	0.317	1.000
	Resting time [sec]	0.310	0.735	0.255	0.616	3.870	0.026	1.000	0.934	1.000	0.925 0.408	1.000	1.000	0.585		4.558	0.018	1.000	0.027	0.076
	Average speed [cm/sec]	0.342	0.712	0.028	0.868	1.813	0.172	1.000	1.000	1.000	0.449 0.643	1.000	1.000	1.000		1.783	0.184	1.000	0.211	0.839
	Number of rearing, 5 min [#]	0.964	0.387	0.022	0.883	1.574	0.215	1.000	1.000	0.336	0.395 0.678	1.000	1.000	1.000		2.407	0.106	1.000	0.451	0.123
	Number of rearing, 10 min [#]	2.399	0.099	1.945	0.168	1.061	0.352	1.000	0.107	0.094	0.473 0.628	1.000	1.000	1.000		2.987	0.064	1.000	0.302	0.075
	Number of rearing, 15 min [#]	1.178	0.315	4.042	0.049	1.212	0.305	1.000	0.631	0.278	0.842 0.441	0.640	1.000	1.000		1.625	0.212	1.000	0.301	0.565
	Number of rearing, 20 min [#]	1.756	0.181	2.606	0.112	0.482	0.620	1.000	0.186	0.262	0.399 0.675	1.000	1.000	1.000		1.767	0.187	1.000	0.255	0.538
	Total number of rearing [#]	1.933	0.154	2.305	0.134	0.958	0.389	1.000	0.245	0.117	0.222 0.802	1.000	1.000	1.000		2.573	0.092	1.000	0.211	0.159
centre	Distance travelled [cm]	0.737	0.483	2.650	0.109	2.407	0.099	1.000	1.000	0.697	1.818 0.181	0.201	1.000	1.000		1.149	0.329	1.000	0.418	1.000
	Resting time [sec]	0.289	0.750	9.379	0.003	3.284	0.044	1.000	1.000	0.686	0.555 0.580	1.000	0.913	1.000		6.511	0.004	1.000	0.015	0.010
	Permanance time [sec]	1.466	0.239	4.683	0.034	1.300	0.280	0.525	1.000	0.504	2.454 0.104	0.133	1.000	0.361		0.044	0.957	1.000	1.000	1.000
	Average speed [cm/sec]	0.228	0.797	0.535	0.467	1.530	0.225	1.000	1.000	1.000	0.386 0.684	1.000	1.000	1.000		1.280	0.291	0.684	0.399	1.000
	% Distance travelled, 5 min [%]	1.434	0.246	0.492	0.486	1.225	0.301	0.451	1.000	0.631	1.869 0.173	0.239	1.000	0.483		0.014	0.986	1.000	1.000	1.000
	% Distance travelled, 10 min [%]	1.553	0.220	0.879	0.352	1.193	0.310	0.696	1.000	0.363	1.810 0.182	0.354	1.000	0.328		0.104	0.901	1.000	1.000	1.000
	% Distance travelled, 15 min [%]	0.242	0.786	1.739	0.192	0.464	0.631	1.000	1.000	1.000	0.719 0.496	1.000	1.000	0.767		0.019	0.981	1.000	1.000	1.000
	% Distance travelled, 20 min [%]	0.921	0.404	7.473	0.008	1.378	0.260	0.778	1.000	1.000	2.127 0.138	0.178	0.447	1.000		0.025	0.975	1.000	1.000	1.000
	% Total distance travelled [%]	1.230	0.299	4.191	0.045	1.610	0.208	0.566	1.000	0.798	2.345 0.114	0.133	1.000	0.474		0.021	0.979	1.000	1.000	1.000
	% Time spent in centre, 5 min [%]	1.283	0.284	0.710	0.403	0.559	0.574	0.488	1.000	0.659	1.212 0.313	0.421	1.000	0.942		0.197	0.822	1.000	1.000	1.000
	% Time spent in centre, 10 min [%]	1.347	0.268	1.709	0.196	1.689	0.193	1.000	1.000	0.385	1.958 0.160	0.360	1.000	0.257		0.335	0.718	1.000	1.000	1.000
	% Time spent in centre, 15 min [%]	0.374	0.690	1.983	0.164	0.841	0.436	1.000	1.000	1.000	1.259 0.300	0.787	1.000	0.426		0.103	0.903	1.000	1.000	1.000
	% Time spent in centre, 20 min [%]	1.234	0.298	8.181	0.006	1.138	0.327	0.476	1.000	1.000	1.999 0.154	0.196	0.510	1.000		0.185	0.832	1.000	1.000	1.000
	% Total time spent in centre [%]	1.455	0.241	4.677	0.035	1.296	0.281	0.528	1.000	0.511	2.444 0.105	0.134	1.000	0.365		0.042	0.959	1.000	1.000	1.000
	Latency to enter in the centre [sec]	1.213	0.304	0.351	0.556	1.272	0.288	0.682	1.000	0.677	1.654 0.209	1.000	0.659	0.253		0.747	0.482	0.756	1.000	1.000
	Number of entries in the centre [#]	1.156	0.322	0.084	0.773	1.269	0.289	1.000	0.530	0.395	0.349 0.708	1.000	1.000	1.000		2.378	0.108	1.000	0.129	0.440
periphery	Distance travelled [cm]	0.410	0.665	0.516	0.475	0.821	0.445	1.000	1.000	1.000	0.048 0.953	1.000	1.000	1.000		1.125	0.337	1.000	0.445	1.000
	Resting time [sec]	0.502	0.608	0.324	0.571	3.182	0.048	1.000	1.000	1.000	1.125 0.339	1.000	1.000	0.434		2.879	0.070	1.000	0.088	0.279
	Permanance time [sec]	1.466	0.239	4.683	0.034	1.300	0.280	0.525	1.000	0.504	2.454 0.104	0.133	1.000	0.361		0.044	0.957	1.000	1.000	1.000
	Average speed [cm/sec]	0.386	0.682	0.056	0.813	1.650	0.201	1.000	1.000	0.991	0.547 0.585	1.000	1.000	1.000		1.556	0.226	1.000	0.275	0.866

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A21: ANOVA table of OF testing of young Pink1 x DAT mice

		Pink1 x DAT, young, OF									males					females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value				Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
whole area	Distance travelled, 5 min [cm]	1.160	0.320	19.398	0.00004	0.108	0.898	0.343	1.000	0.488	0.473	0.628	1.000	1.000	1.000	0.691	0.508	0.953	1.000	0.905
	Distance travelled, 10 min [cm]	0.271	0.763	10.149	0.002	2.357	0.103	1.000	1.000	1.000	1.634	0.211	0.797	0.247	1.000	1.106	0.343	0.497	0.882	1.000
	Distance travelled, 15 min [cm]	0.592	0.556	11.866	0.001	1.035	0.361	0.846	1.000	0.785	0.887	0.422	1.000	0.819	0.739	0.753	0.479	0.769	1.000	1.000
	Distance travelled, 20 min [cm]	0.075	0.928	6.845	0.011	0.259	0.773	1.000	1.000	1.000	0.086	0.918	1.000	1.000	1.000	0.227	0.798	1.000	1.000	1.000
	Total distance travelled [cm]	0.630	0.536	16.952	0.0001	0.665	0.518	0.797	1.000	0.753	0.470	0.629	1.000	1.000	1.000	0.725	0.492	0.714	1.000	1.000
	Resting time [sec]	1.016	0.368	0.031	0.860	0.409	0.666	1.000	1.000	0.506	0.538	0.589	1.000	1.000	1.000	0.838	0.442	0.894	0.716	1.000
	Average speed [cm/sec]	0.771	0.467	15.164	0.0002	0.678	0.511	0.756	1.000	0.611	0.553	0.580	1.000	1.000	1.000	0.783	0.466	0.673	1.000	0.563
	Number of rearing, 5 min [#]	1.369	0.262	2.629	0.110	1.565	0.217	0.299	1.000	1.000	0.088	0.916	1.000	1.000	1.000	2.653	0.086	0.098	1.000	0.307
	Number of rearing, 10 min [#]	0.603	0.550	0.089	0.767	3.050	0.054	1.000	1.000	1.000	1.341	0.276	0.575	0.426	1.000	2.646	0.087	0.090	1.000	0.407
	Number of rearing, 15 min [#]	0.510	0.603	0.002	0.968	1.294	0.281	1.000	1.000	0.998	1.169	0.324	1.000	0.430	0.985	0.599	0.556	0.869	1.000	1.000
	Number of rearing, 20 min [#]	0.344	0.710	0.066	0.799	1.104	0.338	1.000	1.000	1.000	0.926	0.407	0.979	0.598	1.000	0.505	0.609	0.995	1.000	1.000
	Total number of rearing [#]	0.655	0.523	0.185	0.669	1.978	0.147	1.000	1.000	0.852	0.734	0.488	1.000	0.735	1.000	2.022	0.150	0.166	1.000	0.592
centre	Distance travelled [cm]	0.701	0.500	2.619	0.111	0.600	0.552	1.000	0.727	1.000	0.029	0.971	1.000	1.000	1.000	1.076	0.353	1.000	1.000	1.000
	Resting time [sec]	1.098	0.340	0.159	0.691	0.359	0.700	0.675	1.000	0.615	0.525	0.597	0.940	1.000	1.000	0.876	0.427	1.000	0.588	0.435
	Permanance time [sec]	0.031	0.969	0.107	0.745	2.203	0.119	1.000	1.000	1.000	0.934	0.403	0.904	0.615	1.000	1.273	0.294	0.714	1.000	1.000
	Average speed [cm/sec]	0.091	0.914	9.094	0.004	1.833	0.168	1.000	1.000	1.000	1.662	0.206	0.492	0.289	1.000	0.485	0.620	1.000	1.000	1.000
	% Distance travelled, 5 min [%]	0.496	0.612	0.299	0.586	1.838	0.168	1.000	0.943	1.000	0.280	0.758	1.000	1.000	1.000	2.178	0.130	0.296	0.212	1.000
	% Distance travelled, 10 min [%]	0.916	0.405	0.787	0.378	2.264	0.112	1.000	0.478	1.000	0.398	0.675	1.000	1.000	1.000	3.061	0.061	0.200	0.085	1.000
	% Distance travelled, 15 min [%]	0.680	0.510	0.169	0.683	0.197	0.822	1.000	0.726	1.000	0.072	0.930	1.000	1.000	1.000	0.841	0.441	1.000	0.616	1.000
	% Distance travelled, 20 min [%]	0.230	0.795	0.259	0.613	0.758	0.473	1.000	1.000	1.000	0.204	0.817	1.000	1.000	1.000	0.835	0.443	0.648	1.000	1.000
	% Total distance travelled [%]	0.891	0.415	0.453	0.504	1.654	0.199	1.000	0.645	0.645	0.066	0.937	1.000	1.000	1.000	2.449	0.103	0.214	0.187	1.000
	% Time spent in centre, 5 min [%]	0.105	0.901	0.418	0.520	2.236	0.115	1.000	1.000	1.000	0.947	0.398	1.000	0.535	1.000	1.391	0.264	0.614	0.400	1.000
	% Time spent in centre, 10 min [%]	0.412	0.664	0.218	0.642	2.925	0.061	1.000	1.000	1.000	0.997	0.380	0.612	0.772	1.000	2.336	0.113	0.870	0.116	0.998
	% Time spent in centre, 15 min [%]	0.106	0.899	0.495	0.485	0.882	0.419	1.000	1.000	1.000	0.241	0.787	1.000	1.000	1.000	0.768	0.473	1.000	0.674	1.000
	% Time spent in centre, 20 min [%]	0.418	0.660	0.142	0.708	0.483	0.619	1.000	1.000	1.000	0.232	0.794	1.000	1.000	1.000	0.604	0.553	1.000	1.000	0.938
	% Total time spent in centre [%]	0.031	0.970	0.106	0.746	2.213	0.118	1.000	1.000	1.000	0.937	0.402	0.900	0.613	1.000	1.280	0.292	0.715	0.430	1.000
	Latency to enter in the centre [sec]	3.835	0.027	0.034	0.854	0.497	0.611	0.144	1.000	0.035	1.635	0.211	0.809	1.000	0.251	2.245	0.123	0.295	0.168	0.561
	Number of entries in the centre [#]	0.531	0.590	6.501	0.013	0.746	0.478	1.000	1.000	1.000	0.013	0.987	1.000	1.000	1.000	1.006	0.377	1.000	0.935	0.000
periphery	Distance travelled [cm]	1.160	0.320	19.592	0.00004	2.016	0.142	0.269	1.000	0.727	0.717	0.496	1.000	0.815	1.000	1.995	0.153	0.185	1.000	1.000
	Resting time [sec]	0.917	0.405	0.149	0.701	0.849	0.432	1.000	1.000	0.644	1.012	0.375	1.000	0.499	1.000	0.681	0.514	0.911	0.970	0.435
	Permanance time [sec]	0.031	0.969	0.107	0.745	2.203	0.119	1.000	1.000	1.000	0.934	0.404	0.904	0.615	1.000	1.273	0.294	0.714	1.000	1.000
	Average speed [cm/sec]	1.000	0.374	16.776	0.0001	0.387	0.680	0.400	1.000	0.636	0.285	0.754	1.000	1.000	1.000	0.900	0.417	0.576	1.000	0.479

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A22: ANOVA table of OF testing of mid-aged Pink1 x DAT mice

		Pink1 x DAT, mid-aged, OF									males					females				
		Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype	Post hoc Bonferroni, p-value				Genotype	Post hoc Bonferroni, p-value			
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2		F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
whole area	Distance travelled, 5 min [cm]	3.175	0.049	5.737	0.020	0.034	0.967	1.000	0.313	0.035	3.272 0.053	1.000	0.288	0.055		0.971	0.390	1.000	1.000	0.538
	Distance travelled, 10 min [cm]	4.206	0.020	0.136	0.714	0.880	0.420	0.188	1.000	0.016	2.930 0.070	0.098	1.000	0.221		2.501	0.099	1.000	0.516	0.108
	Distance travelled, 15 min [cm]	3.000	0.058	0.097	0.757	0.367	0.694	0.665	0.655	0.046	0.766 0.474	1.000	1.000	0.683		2.393	0.109	0.898	0.797	0.111
	Distance travelled, 20 min [cm]	2.905	0.063	0.149	0.701	1.573	0.216	0.589	0.818	0.055	0.529 0.595	1.000	1.000	1.000		3.166	0.057	0.247	1.000	0.061
	Total distance travelled [cm]	3.878	0.026	0.618	0.435	0.290	0.749	0.445	0.527	0.018	1.634 0.213	1.000	1.000	0.246		2.366	0.111	0.832	0.876	0.113
	Resting time [sec]	4.458	0.016	4.625	0.036	0.218	0.805	1.000	0.138	0.025	2.969 0.068	1.000	0.245	0.082		1.535	0.232	1.000	0.782	0.295
	Average speed [cm/sec]	4.203	0.020	0.442	0.509	0.279	0.758	0.464	0.415	0.013	1.870 0.173	1.000	1.000	0.190		2.490	0.100	0.853	0.784	0.101
	Number of rearing, 5 min [#]	1.301	0.280	0.001	0.976	1.081	0.346	1.000	1.000	0.382	2.082 0.144	1.000	0.242	0.282		0.565	0.574	0.902	1.000	1.000
	Number of rearing, 10 min [#]	4.192	0.020	5.829	0.019	0.455	0.637	1.000	0.195	0.023	1.105 0.345	1.000	1.000	0.449		3.708	0.036	1.000	0.157	0.047
	Number of rearing, 15 min [#]	2.313	0.108	2.732	0.104	0.595	0.555	1.000	0.476	0.140	0.280 0.758	1.000	1.000	1.000		3.167	0.057	0.899	0.468	0.055
	Number of rearing, 20 min [#]	2.756	0.072	5.295	0.025	0.974	0.384	0.984	0.724	0.100	1.195 0.318	1.000	0.490	0.682		2.414	0.107	0.290	1.000	0.139
	Total number of rearing [#]	3.587	0.034	3.738	0.058	0.419	0.660	1.000	0.378	0.042	1.234 0.306	1.000	0.617	0.495		2.806	0.076	0.499	1.000	0.075
centre	Distance travelled [cm]	3.280	0.045	0.469	0.496	0.981	0.381	0.644	0.592	0.038	0.714 0.499	1.000	1.000	0.817		2.842	0.074	0.979	0.534	0.074
	Resting time [sec]	4.766	0.012	2.089	0.154	0.357	0.701	1.000	0.089	0.014	1.701 0.201	1.000	1.000	0.227		3.484	0.044	1.000	0.118	0.076
	Permanance time [sec]	2.884	0.064	4.597	0.036	0.851	0.432	0.227	1.000	0.114	0.377 0.689	1.000	1.000	1.000		3.393	0.047	0.202	1.000	0.052
	Average speed [cm/sec]	0.891	0.416	2.089	0.154	0.164	0.850	1.000	0.503	1.000	0.205 0.816	1.000	1.000	1.000		0.751	0.481	1.000	0.692	1.000
	% Distance travelled, 5 min [%]	1.456	0.241	2.624	0.111	1.700	0.192	1.000	0.321	0.871	1.046 0.365	0.565	0.794	1.000		2.527	0.097	0.942	0.691	0.098
	% Distance travelled, 10 min [%]	0.829	0.441	4.637	0.035	0.439	0.647	1.000	1.000	0.709	0.128 0.881	1.000	1.000	1.000		0.987	0.384	1.000	1.000	0.525
	% Distance travelled, 15 min [%]	2.991	0.058	3.021	0.088	0.432	0.651	0.133	1.000	0.137	1.425 0.257	0.316	1.000	0.985		1.978	0.156	0.532	1.000	0.182
	% Distance travelled, 20 min [%]	1.504	0.231	5.666	0.021	2.287	0.111	0.387	1.000	0.785	0.316 0.731	1.000	1.000	1.000		4.415	0.021	0.066	1.000	0.030
	% Total distance travelled [%]	1.794	0.175	8.037	0.006	1.503	0.231	0.643	1.000	0.292	0.064 0.938	1.000	1.000	1.000		3.123	0.059	0.347	1.000	0.058
	% Time spent in centre, 5 min [%]	1.404	0.254	0.579	0.450	1.024	0.365	1.000	0.419	0.662	0.969 0.392	1.000	0.564	1.000		1.621	0.215	0.853	1.000	0.249
	% Time spent in centre, 10 min [%]	1.220	0.303	1.449	0.234	0.090	0.914	0.466	1.000	0.837	0.410 0.667	1.000	1.000	1.000		0.823	0.449	0.679	1.000	1.000
	% Time spent in centre, 15 min [%]	2.902	0.063	4.125	0.047	1.281	0.286	0.191	1.000	0.112	1.921 0.165	0.180	1.000	1.000		2.350	0.113	1.000	0.596	0.122
	% Time spent in centre, 20 min [%]	2.922	0.062	5.040	0.029	2.139	0.127	0.109	1.000	0.243	0.414 0.665	1.000	1.000	1.000		5.194		0.043	1.000	0.016
	% Total time spent in centre [%]	2.892	0.063	4.587	0.036	0.846	0.434	0.223	1.000	0.114	0.382 0.686	1.000	1.000	1.000		3.389	0.047	0.201	1.000	0.052
	Latency to enter in the centre [sec]	0.864	0.427	1.176	0.283	0.297	0.744	1.000	1.000	0.552	0.503 0.610	1.000	1.000	1.000		0.765	0.474	0.793	1.000	0.948
	Number of entries in the centre [#]	3.744	0.030	0.435	0.512	0.441	0.645	0.808	0.363	0.027	1.530 0.234	1.000	0.681	0.303		2.389	0.109	0.831	0.863	0.111
periphery	Distance travelled [cm]	3.247	0.046	3.073	0.085	0.057	0.944	0.493	0.679	0.031	1.806 0.183	1.000	0.919	0.204		1.578	0.223	0.893	1.000	0.260
	Resting time [sec]	2.470	0.093	3.845	0.055	0.686	0.508	1.000	0.445	0.157	2.316 0.117	1.000	0.281	0.172		0.474	0.627	1.000	1.000	1.000
	Permanance time [sec]	2.885	0.064	4.598	0.036	0.851	0.432	0.227	1.000	0.114	0.377 0.689	1.000	1.000	1.000		3.393	0.047	0.202	1.000	0.052
	Average speed [cm/sec]	4.519	0.015	0.701	0.406	0.249	0.781	0.324	0.486	0.010	2.089 0.143	0.952	0.975	0.152		2.588	0.092	0.642	0.971	0.091

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A23: Summary of results of selected OF parameters of young and mid-aged Pink1 x Pet and Pink1 x DAT mice

Total distance travelled [cm]

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	13	23340.81	4817.77	female	ctrl. 1	14	24820.91	4660.15	males	ctrl. 1	11	21219.28	4886.66	females	ctrl. 1	13	20314.08	4604.99
	mt	12	21127.23	3500.45		mt	11	22002.96	5253.34		mt	11	19215.33	3778.88		mt	10	22247.24	5293.75
	ctrl. 2	9	24151.16	4662.74		ctrl. 2	14	22855.26	7061.18		ctrl. 2	9	20758.98	7158.17		ctrl. 2	13	18189.26	7087.52

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	17948.42	4029.60	female	ctrl. 1	10	21533.21	5292.74	males	ctrl. 1	11	14490.86	4892.82	females	ctrl. 1	10	14307.15	4992.46
	mt	11	17761.28	4561.77		mt	12	24246.79	5577.95		mt	10	16249.95	3205.91		mt	11	17276.85	5321.80
	ctrl. 2	12	19239.37	3459.63		ctrl. 2	12	23115.30	4918.31		ctrl. 2	10	17753.65	4060.27		ctrl. 2	12	20027.43	7538.04

Total number of rearing [#]

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	13	147.77	29.99	females	ctrl. 1	14	162.07	34.83	males	ctrl. 1	11	132.82	31.45	females	ctrl. 1	13	124.23	44.00
	mt	12	157.58	22.28		mt	11	145.91	33.83		mt	11	125.82	24.53		mt	10	124.20	33.51
	ctrl. 2	9	148.00	46.73		ctrl. 2	14	124.07	33.17		ctrl. 2	9	123.67	41.78		ctrl. 2	13	91.54	44.29

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	115.42	37.09	females	ctrl. 1	10	100.60	33.01	males	ctrl. 1	11	72.36	37.46	females	ctrl. 1	10	48.80	19.71
	mt	11	101.64	42.16		mt	12	129.42	41.18		mt	10	73.70	17.67		mt	11	66.91	33.01
	ctrl. 2	12	121.17	39.34		ctrl. 2	12	119.67	25.10		ctrl. 2	10	91.50	32.60		ctrl. 2	12	78.33	31.90

% Time spent in centre [%]

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	13	29.40	5.65	females	ctrl. 1	14	31.96	8.93	males	ctrl. 1	11	31.06	10.72	females	ctrl. 1	13	21.60	5.14
	mt	12	29.29	8.90		mt	11	30.39	10.67		mt	11	22.82	4.48		mt	10	21.70	7.76
	ctrl. 2	9	22.24	7.40		ctrl. 2	14	26.26	10.94		ctrl. 2	9	24.47	11.28		ctrl. 2	13	20.77	11.69

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	18.58	8.57	females	ctrl. 1	10	20.17	13.14	males	ctrl. 1	11	18.13	8.76	females	ctrl. 1	10	9.83	6.89
	mt	11	22.38	11.42		mt	12	15.25	6.77		mt	10	21.28	9.30		mt	11	17.01	7.55
	ctrl. 2	12	17.71	5.01		ctrl. 2	12	21.11	8.38		ctrl. 2	10	20.57	8.05		ctrl. 2	12	19.16	10.64

Centre resting time [sec]

Pink1 x Pet

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	13	27.43	9.73	females	ctrl. 1	14	37.71	19.26	males	ctrl. 1	11	19.85	8.51	females	ctrl. 1	13	15.24	8.33
	mt	12	41.77	20.62		mt	11	50.76	25.22		mt	11	17.50	11.00		mt	10	15.38	9.41
	ctrl. 2	9	28.32	15.98		ctrl. 2	14	20.00	13.00		ctrl. 2	9	24.10	21.14		ctrl. 2	13	6.03	3.86

Pink1 x DAT

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	12.08	8.56	females	ctrl. 1	10	9.71	7.41	males	ctrl. 1	11	4.55	3.91	females	ctrl. 1	10	1.74	2.13
	mt	11	16.95	13.72		mt	12	13.76	15.10		mt	10	7.36	5.79		mt	11	2.60	3.43
	ctrl. 2	12	14.34	11.55		ctrl. 2	12	16.53	11.66		ctrl. 2	10	10.52	10.98		ctrl. 2	12	9.73	12.54

Table A24: ANOVA table of ASR and PPI testing of young and mid-aged Pink1 x Pet mice

Pink1 x Pet, young, ASR/PPI											males					females				
		Genotype		Sex		Interaction		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
ASR	NS	1.109	0.336	0.292	0.590	0.483	0.619	0.624	1.000	0.629	1.376	0.268	0.390	1.000	0.710	0.121	0.887	1.000	1.000	1.000
	70 [dB]	0.548	0.581	7.893	0.006	1.481	0.235	1.000	1.000	1.000	4.760	0.016	0.017	0.118	1.000	0.290	0.750	1.000	1.000	1.000
	80 [dB]	0.909	0.408	1.871	0.176	2.235	0.115	1.000	0.717	0.764	2.309	0.116	0.156	0.362	1.000	1.473	0.243	0.770	1.000	0.314
	85 [dB]	0.938	0.397	1.196	0.278	1.231	0.299	1.000	0.643	0.949	1.451	0.250	0.462	0.461	1.000	0.924	0.406	1.000	1.000	0.563
	90 [dB]	0.563	0.572	0.919	0.341	1.108	0.336	1.000	1.000	1.000	1.603	0.218	0.250	1.000	1.000	0.311	0.734	1.000	1.000	1.000
	100 [dB]	1.389	0.256	0.358	0.552	0.701	0.500	0.396	0.533	1.000	0.717	0.496	0.826	1.000	1.000	1.322	0.279	0.772	0.358	1.000
	110 [dB]	1.810	0.172	1.057	0.307	0.450	0.640	0.228	0.577	1.000	0.799	0.459	0.695	1.000	1.000	1.449	0.248	0.503	0.368	1.000
	120 [dB]	0.970	0.384	1.675	0.200	0.715	0.493	0.663	0.835	1.000	0.502	0.610	1.000	1.000	1.000	1.177	0.320	1.000	0.406	1.000
PPI	67 [dB]	1.145	0.324	0.018	0.894	0.971	0.384	1.000	0.428	0.667	1.161	0.326	0.671	0.546	1.000	1.096	0.345	1.000	1.000	0.443
	69 [dB]	0.200	0.819	0.162	0.689	0.768	0.468	1.000	1.000	1.000	0.421	0.660	1.000	1.000	1.000	0.636	0.536	1.000	1.000	0.805
	73 [dB]	0.308	0.736	0.016	0.900	0.399	0.673	1.000	1.000	1.000	0.185	0.832	1.000	1.000	1.000	0.624	0.541	1.000	1.000	1.000
	81 [dB]	0.550	0.580	0.870	0.354	0.215	0.807	1.000	0.677	1.000	0.211	0.811	1.000	1.000	1.000	0.614	0.547	1.000	1.000	1.000
	global PPI	0.656	0.522	0.076	0.783	0.678	0.511	1.000	0.626	1.000	0.536	0.590	0.976	1.000	1.000	0.938	0.401	1.000	1.000	0.573

Pink1 x Pet, mid-aged, ASR/PPI											males					females				
		Genotype		Sex		Interaction		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
ASR	NS	0.064	0.938	0.109	0.742	0.033	0.967	1.000	1.000	1.000	0.076	0.927	1.000	1.000	1.000	0.013	0.987	1.000	1.000	1.000
	70 [dB]	1.071	0.349	0.666	0.418	1.170	0.317	1.000	1.000	0.443	0.191	0.827	1.000	1.000	1.000	1.441	0.252	0.676	1.000	0.347
	80 [dB]	0.038	0.963	3.069	0.085	1.092	0.342	1.000	1.000	1.000	1.443	0.253	0.301	1.000	1.000	0.447	0.644	1.000	1.000	1.000
	85 [dB]	0.760	0.472	1.132	0.291	0.734	0.484	1.000	1.000	0.679	1.834	0.178	0.388	1.000	0.302	0.034	0.967	1.000	1.000	1.000
	90 [dB]	2.762	0.071	5.532	0.022	0.447	0.641	0.333	1.000	0.046	3.225	0.054	0.089	1.000	0.157	0.984	0.385	1.000	1.000	0.531
	100 [dB]	3.903	0.025	1.778	0.187	0.876	0.421	0.062	1.000	0.030	2.734	0.082	0.082	1.000	0.646	2.382	0.109	0.844	1.000	0.110
	110 [dB]	3.776	0.028	1.275	0.263	0.114	0.893	0.025	0.394	0.726	2.639	0.089	0.094	0.486	1.000	1.316	0.282	0.344	1.000	1.000
	120 [dB]	4.061	0.022	0.710	0.403	3.385	0.040	0.034	0.063	1.000	9.819	0.001	0.001	0.013	1.000	0.250	0.781	1.000	1.000	1.000
PPI	67 [dB]	3.201	0.048	0.363	0.549	0.690	0.506	0.339	0.046	1.000	2.209	0.128	1.000	0.150	0.397	1.628	0.212	0.349	0.381	1.000
	69 [dB]	3.078	0.053	4.511	0.038	0.610	0.547	0.116	1.000	0.144	0.829	0.447	1.000	1.000	0.663	2.459	0.102	0.165	1.000	0.234
	73 [dB]	2.853	0.065	0.752	0.389	0.834	0.439	0.063	0.950	0.539	0.472	0.628	1.000	1.000	1.000	2.928	0.068	0.079	1.000	0.294
	81 [dB]	2.150	0.125	0.000	0.998	0.700	0.501	0.317	1.000	0.178	0.269	0.766	1.000	1.000	1.000	2.462	0.101	0.207	1.000	0.182
	global PPI	2.869	0.064	1.156	0.287	1.017	0.368	0.061	0.855	0.597	0.379	0.688	1.000	1.000	1.000	3.130	0.057	0.063	1.000	0.296

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A25: ANOVA table of ASR and PPI testing of young and mid-aged Pink1 x DAT mice

Pink1 x DAT, young, ASR/PPI											males					females				
		Genotype		Sex		Interaction		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
ASR	NS	0.244	0.785	0.821	0.368	2.434	0.096	1.000	1.000	1.000	2.193	0.128	0.136	1.000	0.696	0.858	0.434	0.810	0.804	1.000
	70 [dB]	0.593	0.556	1.613	0.209	0.065	0.937	1.000	1.000	0.765	0.812	0.453	1.000	1.000	0.667	0.179	0.837	1.000	1.000	1.000
	80 [dB]	0.425	0.656	0.067	0.796	0.739	0.482	1.000	1.000	1.000	1.036	0.367	1.000	0.480	1.000	0.079	0.924	1.000	1.000	1.000
	85 [dB]	0.016	0.984	0.006	0.941	0.592	0.556	1.000	1.000	1.000	0.345	0.711	1.000	1.000	1.000	0.258	0.775	1.000	1.000	1.000
	90 [dB]	0.090	0.914	0.015	0.904	1.716	0.188	1.000	1.000	1.000	0.539	0.589	1.000	0.923	1.000	1.356	0.273	1.000	0.356	0.850
	100 [dB]	0.107	0.898	0.402	0.528	0.397	0.674	1.000	1.000	1.000	0.069	0.934	1.000	1.000	1.000	0.432	0.653	1.000	1.000	1.000
	110 [dB]	0.190	0.827	0.003	0.958	0.253	0.777	1.000	1.000	1.000	0.228	0.797	1.000	1.000	1.000	0.213	0.809	1.000	1.000	1.000
	120 [dB]	0.018	0.982	0.083	0.774	0.186	0.831	1.000	1.000	1.000	0.130	0.878	1.000	1.000	1.000	0.086	0.918	1.000	1.000	1.000
PPI	67 [dB]	0.921	0.404	0.543	0.464	0.618	0.542	0.830	1.000	0.583	1.203	0.314	1.000	0.828	0.439	0.524	0.597	0.944	1.000	1.000
	69 [dB]	0.330	0.720	0.727	0.397	0.092	0.912	1.000	1.000	1.000	0.483	0.622	1.000	1.000	1.000	0.050	0.951	1.000	1.000	1.000
	73 [dB]	1.014	0.369	2.313	0.133	0.041	0.960	0.638	1.000	0.838	1.006	0.377	0.627	1.000	0.769	0.252	0.779	1.000	1.000	1.000
	81 [dB]	1.693	0.192	1.064	0.306	0.033	0.968	0.281	1.000	0.561	0.992	0.382	0.620	1.000	0.806	0.782	0.466	0.690	1.000	1.000
	global PPI	1.219	0.302	0.561	0.457	0.071	0.931	0.548	1.000	0.565	1.220	0.309	0.751	1.000	0.450	0.384	0.684	1.000	1.000	1.000

Pink1 x DAT, mid-aged, ASR/PPI											males					females				
		Genotype		Sex		Interaction		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value			Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
ASR	NS	1.057	0.354	1.018	0.317	1.473	0.238	1.000	0.499	0.771	0.726	0.493	0.746	1.000	1.000	1.806	0.182	1.000	0.747	0.205
	70 [dB]	4.775	0.012	2.527	0.117	3.023	0.056	0.758	0.009	0.162	1.379	0.268	0.358	0.739	1.000	5.546	0.009	1.000	0.015	0.026
	80 [dB]	4.621	0.014	0.180	0.673	1.356	0.266	0.302	0.012	0.530	4.171	0.026	0.055	0.053	1.000	2.081	0.142	1.000	0.220	0.294
	85 [dB]	6.355	0.003	0.101	0.752	0.010	0.990	0.118	0.002	0.417	2.558	0.095	0.697	0.095	0.963	4.108	0.026	0.232	0.025	0.840
	90 [dB]	4.008	0.023	0.147	0.703	0.006	0.994	0.268	0.019	0.783	2.044	0.148	0.802	0.160	1.000	1.989	0.154	0.596	0.180	1.000
	100 [dB]	3.953	0.025	0.041	0.840	0.130	0.879	0.146	0.026	1.000	3.003	0.066	0.486	0.064	1.000	1.445	0.252	0.503	0.414	1.000
	110 [dB]	1.296	0.281	0.107	0.745	0.841	0.436	0.289	1.000	1.000	0.364	0.698	1.000	1.000	1.000	1.869	0.172	0.216	1.000	0.589
	120 [dB]	1.708	0.190	0.591	0.445	0.308	0.736	0.280	0.373	1.000	0.666	0.522	1.000	0.781	1.000	1.411	0.260	0.324	0.880	1.000
PPI	67 [dB]	1.365	0.263	0.002	0.961	0.396	0.674	0.989	0.327	1.000	0.375	0.691	1.000	1.000	1.000	1.364	0.271	1.000	0.338	0.874
	69 [dB]	0.419	0.660	0.300	0.586	0.229	0.796	1.000	1.000	1.000	0.067	0.935	1.000	1.000	1.000	0.619	0.545	1.000	1.000	0.859
	73 [dB]	0.949	0.393	0.469	0.496	0.312	0.733	1.000	1.000	0.573	0.270	0.765	1.000	1.000	1.000	1.138	0.334	1.000	0.716	0.504
	81 [dB]	0.427	0.655	0.151	0.699	2.408	0.099	1.000	1.000	1.000	0.514	0.604	1.000	1.000	1.000	2.181	0.130	1.000	0.728	0.137
	global PPI	0.837	0.438	0.075	0.785	0.944	0.395	1.000	1.000	0.672	0.002	0.998	1.000	1.000	1.000	2.339	0.114	1.000	0.331	0.143

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1

Table A26: Summary of results of ASR [a.u.] of young and mid-aged Pink1 x Pet mice

	Pink1 x Pet, young																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
stimulus [dB]	mean	SD	n	mean	SD	N	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
NS	0.91	0.25	13	1.11	0.31	12	1.08	0.35	9	1.04	0.20	14	1.07	0.30	11	1.10	0.34	14
70	1.82	0.52	13	2.52	0.70	12	1.96	0.36	9	2.97	1.42	14	2.69	1.40	11	2.63	0.68	14
80	3.50	1.63	13	4.87	2.01	12	3.68	0.78	9	5.67	3.24	14	4.47	2.56	11	4.05	1.21	14
85	5.65	2.84	13	7.76	4.66	12	5.44	2.01	9	8.47	5.05	14	7.09	3.83	11	6.34	2.85	14
90	7.37	3.14	13	10.75	5.75	12	8.91	4.30	9	10.93	5.96	14	10.25	4.52	11	9.33	4.78	14
100	12.59	4.63	13	15.79	7.94	12	15.38	7.97	9	14.90	9.52	14	19.30	10.72	11	13.19	7.16	14
110	13.78	5.80	13	18.02	9.95	12	16.92	8.89	9	16.89	8.94	14	22.22	9.38	11	16.24	8.81	14
120	13.63	4.93	13	16.66	9.81	12	16.71	9.61	9	17.95	11.48	14	21.83	8.69	11	15.77	7.65	14

	Pink1 x Pet, mid-aged																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
stimulus [dB]	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
NS	1.33	0.39	12	1.39	0.26	11	1.38	0.47	9	1.33	0.30	12	1.35	0.34	10	1.33	0.35	13
70	1.41	0.50	12	1.49	0.27	11	1.38	0.42	9	1.93	1.42	12	1.45	0.37	10	1.34	0.38	13
80	1.78	0.73	12	2.47	1.08	11	2.08	0.93	9	3.42	3.56	12	2.44	1.10	10	2.91	1.43	13
85	2.18	0.84	12	3.57	2.57	11	3.77	2.37	9	3.82	3.36	12	3.66	1.77	10	3.95	2.20	13
90	2.44	1.00	12	3.98	1.73	11	3.88	1.85	9	4.03	3.13	12	4.49	2.05	10	5.34	1.27	13
100	3.45	1.03	12	5.81	3.39	11	4.81	1.86	9	4.34	2.69	12	5.58	2.43	10	6.64	2.42	13
110	5.23	2.22	12	8.25	4.16	11	6.19	2.30	9	6.34	3.00	12	8.61	3.72	10	7.41	2.71	13
120	7.43	2.65	12	13.86	4.73	11	8.84	2.28	9	11.30	5.22	12	11.27	4.60	10	10.15	3.33	13

Table A27: Summary of results of ASR [a.u.] of young and mid-aged Pink1 x DAT mice

	young Pink1 x DAT																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
stimulus [dB]	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
NS	0.97	0.13	12	1.24	0.39	11	1.13	0.30	12	1.25	0.50	10	1.07	0.27	12	1.25	0.32	12
70	2.20	1.12	12	2.53	0.76	11	2.67	0.69	12	2.73	1.33	10	2.78	2.10	12	3.10	0.92	12
80	4.71	3.05	12	3.99	1.44	11	5.43	2.03	12	5.05	2.80	10	4.86	2.18	12	4.66	1.69	12
85	6.74	3.48	12	7.00	3.08	11	7.94	4.00	12	7.82	3.60	10	7.31	3.46	12	6.75	2.98	12
90	9.63	4.68	12	8.33	3.26	11	10.63	6.57	12	9.83	5.11	10	10.72	5.38	12	7.59	2.87	12
100	12.67	6.41	12	12.22	3.73	11	13.21	7.39	12	10.98	4.32	10	13.17	7.24	12	10.99	6.50	12
110	13.84	8.54	12	13.75	4.53	11	15.75	9.11	12	13.50	6.23	10	15.71	7.96	12	14.44	8.30	12
120	14.77	8.84	12	15.20	5.67	11	16.36	7.61	12	15.00	9.29	10	15.54	8.77	12	13.98	8.83	12
	mid-aged Pink1 x DAT																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
stimulus [dB]	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
NS	0.98	0.23	11	1.08	0.39	10	0.93	0.16	10	0.94	0.20	10	1.09	0.29	11	1.17	0.30	12
70	1.22	0.41	11	1.46	0.55	10	1.13	0.31	10	1.01	0.29	10	1.74	0.72	11	1.67	0.48	12
80	2.27	1.21	11	3.71	1.48	10	2.25	1.00	10	2.09	0.57	10	3.37	1.83	11	3.24	1.72	12
85	3.18	1.60	11	5.54	2.89	10	4.24	2.19	10	3.39	1.10	10	5.78	2.49	11	4.30	1.63	12
90	4.53	1.73	11	6.75	2.77	10	5.48	2.59	10	4.84	2.71	10	7.02	2.39	11	5.62	2.21	12
100	5.58	1.31	11	8.43	2.84	10	6.71	3.16	10	5.96	3.19	10	8.11	2.96	11	6.20	3.11	12
110	8.54	2.81	11	10.01	3.05	10	9.24	5.04	10	9.41	4.56	10	10.27	4.19	11	7.12	2.73	12
120	11.08	3.87	11	13.38	4.39	10	11.99	4.80	10	10.86	5.51	10	13.08	4.28	11	9.80	3.79	12

Table A28: Summary of results of PPI [a.u.] of young and mid-aged Pink1 x Pet mice

	Pink1 x Pet, young																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
pre stimulus [dB]	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
67	46.09	14.07	13	53.09	13.48	12	44.61	12.51	9	52.67	14.36	14	48.26	14.97	11	44.25	14.16	14
69	48.60	16.05	13	54.00	13.96	12	51.96	11.32	9	53.07	18.31	14	50.40	8.91	11	46.89	12.09	14
73	56.15	14.51	13	59.37	12.74	12	58.16	9.80	9	59.81	15.99	14	60.00	8.82	11	55.06	10.79	14
81	64.69	12.04	13	67.12	7.24	12	64.70	9.75	9	64.32	13.72	14	64.66	7.88	11	60.13	10.92	14
global	53.88	12.62	13	58.40	9.86	12	54.86	8.89	9	57.47	14.05	14	55.83	8.50	11	51.58	9.85	14
	Pink1 x Pet, mid-aged																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
pre stimulus [dB]	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
67	11.95	23.82	12	17.78	22.61	11	-4.90	24.08	9	-2.91	34.42	12	17.53	24.29	10	-1.97	24.62	13
69	26.21	22.35	12	33.51	16.23	11	37.70	20.28	9	5.09	35.68	12	28.59	19.77	10	25.17	19.83	13
73	27.42	28.14	12	38.55	21.99	11	30.00	30.76	9	7.64	40.77	12	39.50	20.47	10	29.44	25.62	13
81	35.68	21.11	12	39.83	27.17	11	43.67	22.17	9	25.54	26.07	12	47.22	21.84	10	46.48	28.10	13
global	25.32	18.53	12	32.42	20.26	11	26.62	19.78	9	8.84	30.41	12	33.21	16.74	10	24.78	16.77	13

Table A29: Summary of results of PPI [a.u.] of young and mid-aged Pink1 x DAT mice

	young Pink1 x DAT																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
pre stimulus [dB]	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
67	39.83	13.43	12	41.84	14.49	11	48.26	11.85	12	42.21	17.84	10	50.24	19.32	12	46.14	15.25	12
69	47.46	13.09	12	52.77	14.52	11	52.82	15.99	12	46.32	16.34	10	47.27	23.63	12	48.84	12.25	12
73	53.31	14.34	12	59.85	9.46	11	59.08	10.54	12	49.55	13.06	10	54.12	14.98	12	53.14	16.26	12
81	50.90	14.95	12	57.41	8.24	11	56.46	10.21	12	46.38	21.17	10	55.02	11.08	12	52.59	14.36	12
global	47.87	10.55	12	52.97	8.87	11	54.15	10.33	12	46.11	14.17	10	51.66	15.61	12	50.18	13.51	12
	mid-aged Pink1 x DAT																	
	males									females								
	ctrl.1			mt			ctrl.2			ctrl.1			mt			ctrl.2		
pre stimulus [dB]	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
67	25.67	22.66	11	18.12	28.93	10	27.17	19.24	10	33.12	13.30	10	15.27	29.68	11	21.63	24.62	12
69	32.66	25.05	11	35.22	20.05	10	31.56	19.49	10	40.84	18.16	10	37.25	21.73	11	30.60	22.45	12
73	43.02	27.45	11	43.47	21.04	10	35.77	26.32	10	52.96	14.50	10	41.69	25.42	11	39.97	19.60	12
81	49.05	22.69	11	55.91	8.36	10	57.02	21.09	10	62.34	18.60	10	50.87	21.80	11	42.66	22.13	12
global	37.60	20.58	11	38.18	16.40	10	37.88	19.03	10	47.32	13.79	10	36.27	16.89	11	33.71	13.07	12

Table A30: Description of CW parameters

1	Cadence [steps/sec]	Steps per sec
2	Stance duration [sec]	Duration of contact of a paw with the glass plate during one paw placement
3	Stand [sec]	Duration of contact with the glass plate
4	Stand index [cm/sec]	Speed at which paw looses contact with glass plate
5	Body speed [cm/sec]	Speed of the body from one initial contact of a paw to the next initial contact
6	Body speed variation [%]	The absolute difference between the body speed and the average speed of a run divided by the average speed
7	Number of steps [#]	Total number of selected steps
8	Swing duration [sec]	Duration of no contact of a paw with the glass plate
9	Swing speed [cm/sec]	Speed of paw during Swing
10	Run duration [sec]	The duration of the recorded run
11	Run speed [cm/sec]	The average speed of the recorded run, which is the speed of the animal´s body
12	Run maximum variation [%]	The maximum variation in walking speed in the recorded run
13	Stride length [cm]	Distance between successive placements of the same paw
14	Step cycle [sec]	Time between two consecutive initial contacts of the same paw
15	Duty cycle [%]	Stand as percentage of step cycle
16	Base of support [cm]	Average width between front paws or hind paws
17	Initial dual stance [sec]	Duration of groud contact for both hind paws simultaneously
18	Terminal dual stance [sec]	The second step in a step cycle of a hind paw that the contralateral hind paw also makes contact with the glass plate
19	Print position right paws [cm]	Distance between the position of the hind paw and the position of the previously placed right front paw on the same side of the body
20	Print position left paws [cm]	Distance between the position of the hind paw and the position of the previously placed left front paw on the same side of the body
21	Number of patterns	The number of footfall patterns
22	Regularity index [%]	Number of normal step sequence pattern relative to the total number of paw placements
23	Phase dispersion [%]	Temporal relatioship between placement of two paws within the step cycle
24	Couplings [%]	Temporal relatioship between placement of two paws within the step cycle
25	% Max contact at [%]	Duration since the start of the run that a paw makes maximum contact with the glass plate in relation to stand of paw
26	Max contact max intensity [pixel]	Maximum intensity of a paw at maximum contact
27	Max contact mean intensity [pixel]	Mean intensity of a paw at maximum contact
28	Maximum intensity [pixel]	Maximum intensity of complete paw
29	% Maximum intensity at [sec]	Time since the start of the run that the maximum intensity is measured in relative to stand
30	Minimum intensity [pixel]	Minimum intensity of complete paw
31	Mean intensity mean [pixel]	Mean intensity of the mean intensity of the complete paw
32	Mean intensity of 15 most intense pixel [pixel]	Mean of the 15 most intense pixel of paws with highest intensity
33	Print length [cm]	The lenght of complete paw print
34	Print Width [cm]	The width of complete paw print
35	Print area [cm]	The surface area of the complete paw print

* Information are taken from the Reference Manual of CatWalk XT Version 10.5

Table A31: ANOVA table (1) of CW testing of young Pink1 x Pet mice: temporal parameters

				Pink1 x Pet, young, CW										males						females					
				Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
				F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
temporal parameters	1	Cadence [steps/sec]		0.332	0.719	0.011	0.917	3.321	0.043	1.000	1.000	1.000	2.313	0.118	1.000	0.154	0.321	1.268	0.294	1.000	0.894	0.396
	2	Stance duration [sec]	RF	0.100	0.905	0.032	0.859	3.080	0.053	1.000	1.000	1.000	1.834	0.179	1.000	0.202	0.755	1.237	0.303	1.000	0.379	1.000
			RH	0.155	0.857	0.108	0.744	2.493	0.091	1.000	1.000	1.000	1.656	0.210	1.000	0.243	0.834	0.889	0.420	1.000	0.577	1.000
			LF	0.434	0.650	0.756	0.388	1.673	0.196	1.000	1.000	0.782	0.763	0.476	1.000	0.724	1.000	1.507	0.236	1.000	0.628	0.337
			LH	0.289	0.750	7.777	0.007	2.116	0.129	1.000	1.000	1.000	1.508	0.239	1.000	0.602	0.324	0.752	0.479	1.000	1.000	0.708
			FP	0.102	0.903	0.329	0.569	2.491	0.091	1.000	1.000	1.000	1.340	0.279	1.000	0.340	1.000	1.332	0.277	1.000	0.449	0.609
			HP	0.228	0.797	3.288	0.075	2.337	0.105	1.000	1.000	1.000	1.837	0.179	1.000	0.258	0.400	0.645	0.531	1.000	1.000	1.000
	3	Stand [sec]	RF	0.722	0.490	0.355	0.554	2.671	0.077	1.000	1.000	1.000	2.294	0.120	1.000	0.229	0.201	0.601	0.554	1.000	1.000	0.844
			RH	0.264	0.769	1.220	0.274	0.993	0.376	1.000	1.000	1.000	0.955	0.398	1.000	0.593	0.909	0.159	0.854	1.000	1.000	1.000
			LF	0.368	0.694	0.059	0.809	2.234	0.116	1.000	1.000	1.000	1.546	0.231	1.000	0.362	0.461	1.016	0.372	1.000	1.000	0.493
			LH	0.378	0.687	7.933	0.007	1.399	0.255	1.000	1.000	1.000	1.326	0.282	1.000	0.815	0.365	0.241	0.787	1.000	1.000	1.000
			FP	0.495	0.612	0.183	0.670	2.492	0.091	1.000	1.000	1.000	1.936	0.164	1.000	0.279	0.300	0.829	0.445	1.000	1.000	0.624
			HP	0.369	0.693	3.915	0.052	1.167	0.318	1.000	1.000	1.000	1.229	0.308	1.000	0.593	0.501	0.134	0.875	1.000	1.000	1.000
	4	Stand index [cm/sec]	RF	0.529	0.592	0.002	0.964	1.305	0.279	0.973	1.000	1.000	0.714	0.499	1.000	0.780	1.000	1.256	0.297	1.000	1.000	0.383
			RH	0.999	0.374	0.412	0.523	1.344	0.268	0.850	0.724	1.000	1.938	0.163	0.985	0.178	0.833	0.290	0.750	1.000	1.000	1.000
			LF	0.603	0.550	0.153	0.697	3.119	0.051	1.000	1.000	0.459	0.864	0.433	1.000	0.617	1.000	3.609	0.038	1.000	0.122	0.061
			LH	0.682	0.509	2.189	0.144	1.935	0.153	1.000	1.000	1.000	2.704	0.085	1.000	0.198	0.125	0.220	0.804	1.000	1.000	1.000
			FP	0.681	0.510	0.032	0.858	2.725	0.073	0.950	1.000	0.596	1.122	0.340	0.890	0.468	1.000	2.680	0.083	1.000	0.307	0.107
			HP	0.887	0.417	1.658	0.203	2.244	0.115	1.000	0.783	1.000	2.891	0.073	1.000	0.102	0.186	0.328	0.723	1.000	1.000	1.000
	5	Body speed [cm/sec]	RF	0.675	0.513	0.124	0.726	2.917	0.062	1.000	1.000	1.000	2.602	0.093	1.000	0.325	0.107	1.026	0.369	0.746	1.000	0.640
			RH	0.879	0.420	0.134	0.716	2.547	0.087	1.000	0.865	1.000	2.417	0.108	1.000	0.251	0.156	0.991	0.381	0.715	1.000	0.711
			LF	0.545	0.583	0.135	0.715	2.773	0.070	1.000	1.000	1.000	2.497	0.101	1.000	0.307	0.125	0.807	0.454	1.000	1.000	0.695
			LH	0.663	0.519	0.247	0.621	2.567	0.085	1.000	1.000	1.000	2.379	0.112	1.000	0.312	0.144	0.711	0.498	1.000	1.000	0.820
			FP	0.606	0.549	0.130	0.720	2.840	0.066	1.000	1.000	1.000	2.558	0.096	1.000	0.315	0.115	0.903	0.415	0.939	1.000	0.668
			HP	0.765	0.470	0.190	0.664	2.578	0.084	1.000	1.000	1.000	2.417	0.108	1.000	0.276	0.147	0.852	0.435	0.895	1.000	0.756
	6	Body speed variation [%]	RF	1.611	0.208	3.845	0.054	0.979	0.381	0.247	1.000	1.000	2.146	0.136	0.382	0.174	1.000	0.926	0.406	0.832	1.000	0.701
			RH	1.928	0.154	1.201	0.277	0.822	0.444	0.165	1.000	0.852	1.951	0.162	0.313	0.261	1.000	1.117	0.339	0.692	1.000	0.581
			LF	2.065	0.135	2.092	0.153	0.811	0.449	0.167	0.751	1.000	2.991	0.067	0.110	0.140	1.000	0.469	0.629	1.000	1.000	1.000
			LH	1.080	0.346	3.720	0.058	1.941	0.152	0.560	1.000	1.000	1.804	0.184	0.974	0.205	0.940	1.008	0.375	1.000	1.000	0.519
			FP	2.090	0.132	3.381	0.071	0.946	0.394	0.156	0.813	1.000	3.122	0.060	0.135	0.095	1.000	0.731	0.489	0.956	1.000	0.901
			HP	1.838	0.168	2.708	0.105	1.583	0.214	0.196	0.993	1.000	2.111	0.141	0.454	0.168	1.000	1.338	0.275	0.703	1.000	0.404
	7	Number of steps [#]		0.165	0.848	0.205	0.652	1.880	0.161	1.000	1.000	1.000	1.298	0.290	0.590	1.000	0.521	0.810	0.453	0.640	1.000	1.000
	8	Swing duration [sec]	RF	0.493	0.613	2.459	0.122	0.935	0.398	1.000	1.000	0.710	0.586	0.564	0.999	1.000	1.000	0.962	0.392	1.000	0.923	0.612
			RH	0.278	0.758	3.979	0.050	0.999	0.374	1.000	1.000	1.000	0.356	0.704	1.000	1.000	1.000	0.992	0.381	1.000	1.000	0.504
			LF	0.050	0.952	0.805	0.373	2.245	0.114	1.000	1.000	1.000	1.289	0.292	1.000	0.360	1.000	1.038	0.365	0.952	0.491	1.000
			LH	0.252	0.778	17.030	0.000	1.588	0.213	1.000	1.000	0.947	1.351	0.276	0.445	0.532	1.000	0.747	0.481	1.000	0.739	1.000
			FP	0.072	0.930	1.691	0.198	1.613	0.208	1.000	1.000	1.000	0.907	0.416	1.000	0.600	1.000	0.883	0.423	1.000	0.635	1.000
			HP	0.339	0.714	10.548	0.002	1.149	0.324	1.000	1.000	0.852	0.793	0.463	0.996	0.735	1.000	0.839	0.441	1.000	1.000	0.707
	9	Swing speed [cm/sec]	RF	0.304	0.739	2.272	0.137	1.544	0.222	1.000	1.000	1.000	1.006	0.379	1.000	0.944	0.540	0.943	0.399	0.782	1.000	0.716
			RH	0.174	0.841	5.032	0.028	3.116	0.051	1.000	1.000	1.000	2.033	0.151	1.000	0.988	0.162	1.515	0.234	0.543	1.000	0.367
			LF	0.422	0.658	1.210	0.276	2.031	0.140	1.000	1.000	1.000	2.265	0.123	1.000	0.529	0.134	0.316	0.731	1.000	1.000	1.000
			LH	0.249	0.781	20.976	0.000	1.703	0.190	1.000	1.000	1.000	1.309	0.287	1.000	0.374	0.817	0.635	0.536	1.000	1.000	0.841
			FP	0.214	0.808	1.797	0.185	1.802	0.173	1.000	1.000	1.000	1.732	0.196	1.000	0.650	0.235	0.534	0.591	1.000	1.000	0.968
			HP	0.251	0.779	14.686	0.000	2.434	0.096	1.000	1.000	1.000	1.802	0.184	1.000	0.404	0.269	1.137	0.332	1.000	1.000	0.437
	10	Run duration [sec]		0.386	0.682	0.019	0.890	1.790	0.176	1.000	1.000	1.000	1.976	0.158	1.000	0.765	0.172	0.339	0.715	1.000	1.000	1.000
	11	Run speed [cm/sec]		0.527	0.593	0.184	0.670	2.827	0.067	1.000	1.000	1.000	2.574	0.095	1.000	0.375	0.105	0.724	0.492	1.000	1.000	0.763
	12	Run maximum variation [%]		0.478	0.622	4.677	0.034	0.329	0.721	1.000	1.000	1.000	0.037	0.963	1.000	1.000	1.000	0.915	0.410	0.656	0.779	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A32: ANOVA table (2) of CW testing of young Pink1 x Pet mice: comparative paws

			Pink1 x Pet, young, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
comparative paws	13 Stride length [cm]	RF	0.684	0.508	0.412	0.523	1.639	0.202	1.000	1.000	0.982	1.558	0.229	0.514	1.000	0.386	0.756	0.477	0.831	0.859	1.000
		RH	0.868	0.425	0.647	0.424	1.621	0.206	1.000	1.000	0.742	1.637	0.213	0.454	1.000	0.379	0.793	0.460	0.930	0.726	1.000
		LF	0.552	0.579	0.373	0.544	1.481	0.235	1.000	1.000	1.000	1.326	0.282	0.653	1.000	0.459	0.613	0.548	0.911	1.000	1.000
		LH	0.567	0.570	0.282	0.597	1.388	0.257	1.000	1.000	1.000	1.222	0.310	0.722	1.000	0.501	0.632	0.537	0.913	1.000	1.000
		FP	0.616	0.543	0.395	0.532	1.556	0.219	1.000	1.000	1.000	1.437	0.255	0.581	1.000	0.422	0.679	0.514	0.872	0.979	1.000
		HP	0.713	0.494	0.451	0.504	1.498	0.232	1.000	1.000	0.925	1.421	0.259	0.578	1.000	0.434	0.704	0.501	0.919	0.872	1.000
	14 Step cycle [sec]	RF	0.357	0.701	0.079	0.779	2.287	0.110	1.000	1.000	1.000	1.596	0.221	1.000	0.303	0.539	0.936	0.402	1.000	1.000	0.545
		RH	0.261	0.771	0.175	0.677	2.496	0.091	1.000	1.000	1.000	1.646	0.212	1.000	0.277	0.565	1.000	0.378	1.000	1.000	0.549
		LF	0.155	0.856	0.025	0.875	2.380	0.101	1.000	1.000	1.000	1.607	0.219	1.000	0.297	0.544	0.862	0.431	1.000	0.892	0.745
		LH	0.227	0.798	0.076	0.784	2.555	0.086	1.000	1.000	1.000	1.726	0.197	1.000	0.267	0.487	0.909	0.412	1.000	0.949	0.656
		FP	0.242	0.786	0.047	0.830	2.333	0.105	1.000	1.000	1.000	1.608	0.219	1.000	0.298	0.539	0.875	0.426	1.000	1.000	0.634
		HP	0.238	0.789	0.122	0.728	2.541	0.087	1.000	1.000	1.000	1.688	0.204	1.000	0.272	0.520	0.965	0.391	1.000	0.977	0.590
	15 Duty cycle [%]	RF	1.554	0.219	4.535	0.037	1.742	0.184	1.000	0.694	0.366	3.001	0.067	1.000	0.400	0.066	0.049	0.952	1.000	1.000	1.000
		RH	0.286	0.753	3.470	0.067	0.220	0.803	1.000	1.000	1.000	0.285	0.754	1.000	1.000	1.000	0.210	0.812	1.000	1.000	1.000
		LF	0.390	0.679	1.409	0.240	1.577	0.215	1.000	1.000	1.000	0.694	0.508	1.000	1.000	0.759	1.254	0.298	0.475	1.000	0.668
		LH	0.444	0.644	22.394	0.000	1.068	0.350	1.000	1.000	0.806	1.319	0.284	0.638	1.000	0.472	0.239	0.788	1.000	1.000	1.000
		FP	0.654	0.523	3.348	0.072	1.873	0.162	1.000	0.902	1.000	2.003	0.154	1.000	0.641	0.172	0.545	0.585	0.995	1.000	1.000
		HP	0.517	0.599	12.575	0.001	0.233	0.793	1.000	1.000	0.766	0.781	0.468	1.000	1.000	0.669	0.036	0.965	1.000	1.000	1.000
	16 Base of support [cm]	FP	0.556	0.577	0.098	0.755	0.909	0.408	0.825	1.000	1.000	0.069	0.933	1.000	1.000	1.000	2.046	0.144	0.152	0.695	1.000
		HP	0.576	0.565	6.565	0.013	0.749	0.477	1.000	0.741	1.000	0.526	0.597	1.000	1.000	0.985	0.777	0.468	0.664	1.000	1.000
	17 Initial dual stance [sec]	RF	0.220	0.804	0.008	0.928	0.451	0.639	1.000	1.000	1.000	0.528	0.596	1.000	1.000	1.000	0.113	0.893	1.000	1.000	1.000
		RH	0.760	0.472	3.742	0.058	0.050	0.951	1.000	1.000	0.489	0.124	0.884	1.000	1.000	1.000	1.116	0.339	1.000	1.000	0.440
		LF	1.151	0.323	3.479	0.067	2.878	0.064	1.000	0.530	0.808	2.516	0.100	0.784	0.956	0.102	1.175	0.321	0.403	1.000	1.000
		LH	0.051	0.951	0.356	0.553	0.709	0.496	1.000	1.000	1.000	0.310	0.736	1.000	1.000	1.000	0.466	0.631	1.000	1.000	1.000
		FP	0.739	0.482	1.236	0.270	1.696	0.192	1.000	0.860	1.000	1.682	0.205	1.000	0.965	0.233	0.601	0.554	0.846	1.000	1.000
		HP	0.228	0.797	2.021	0.160	0.176	0.839	1.000	1.000	1.000	0.247	0.783	1.000	1.000	1.000	0.127	0.881	1.000	1.000	1.000
	18 Terminal dual stance [sec]	RF	1.117	0.334	3.840	0.055	2.966	0.059	1.000	0.438	1.000	2.182	0.132	1.000	0.790	0.139	1.645	0.207	0.238	1.000	1.000
		RH	0.016	0.985	1.045	0.311	0.964	0.387	1.000	1.000	1.000	0.679	0.516	0.935	1.000	1.000	0.375	0.690	1.000	1.000	1.000
		LF	0.512	0.602	0.020	0.888	0.429	0.653	1.000	1.000	1.000	0.879	0.427	1.000	0.770	0.763	0.056	0.946	1.000	1.000	1.000
		LH	0.767	0.469	2.888	0.094	0.229	0.796	1.000	0.920	0.466	0.067	0.935	1.000	1.000	1.000	1.552	0.226	1.000	0.616	0.320
		FP	1.040	0.359	1.210	0.276	1.743	0.183	1.000	0.553	1.000	1.902	0.169	1.000	0.570	0.199	0.734	0.487	0.704	1.000	1.000
		HP	0.353	0.704	2.794	0.100	0.255	0.775	1.000	1.000	1.000	0.300	0.743	1.000	1.000	1.000	0.307	0.738	1.000	1.000	1.000
	19 Print position right paws [cm]		0.597	0.553	10.049	0.002	2.991	0.058	1.000	1.000	0.575	1.728	0.197	0.228	1.000	0.994	1.818	0.177	0.473	0.217	1.000
	20 Print position left paws [cm]		0.184	0.833	6.639	0.012	2.397	0.099	1.000	1.000	1.000	1.259	0.300	0.489	1.000	0.689	1.176	0.320	0.402	1.000	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A33: ANOVA table (3) of CW testing of young Pink1 x Pet mice: interlimb coordination and individual paw

			Pink1 x Pet, young, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
interlimb coordination	21	Number of patterns	0.722	0.490	0.330	0.567	1.457	0.241	1.000	1.000	0.998	2.155	0.135	0.529	1.000	0.171	0.357	0.702	1.000	1.000	1.000
	22	Regularity index [%]	3.544	0.035	0.026	0.872	0.757	0.473	0.153	1.000	0.027	0.402	0.673	1.000	1.000	1.000	5.366	0.009	0.120	1.000	0.009
	23	Phase dispersion [%] RF-LH	0.712	0.495	3.106	0.083	0.799	0.454	1.000	0.758	0.452	0.784	0.467	0.670	1.000	1.000	1.013	0.373	1.000	0.730	0.668
		LF-RH	0.416	0.661	0.533	0.468	0.149	0.862	1.000	1.000	1.000	0.417	0.663	1.000	1.000	1.000	0.355	0.704	1.000	1.000	1.000
		LH-RH	0.176	0.839	8.924	0.004	2.559	0.086	1.000	1.000	1.000	0.665	0.522	0.796	1.000	1.000	2.217	0.124	0.170	1.000	0.383
		LF-RF	0.252	0.778	1.348	0.250	1.757	0.181	1.000	1.000	1.000	0.436	0.651	1.000	1.000	1.000	1.507	0.236	0.281	1.000	1.000
		RF-RH	1.009	0.370	0.385	0.537	1.929	0.154	0.429	1.000	1.000	0.680	0.515	1.000	1.000	0.901	2.250	0.120	0.175	1.000	0.341
		LF-LH	0.918	0.405	9.601	0.003	0.108	0.898	0.571	1.000	1.000	0.419	0.662	1.000	1.000	1.000	0.621	0.543	0.874	1.000	1.000
	24	Couplings [%] RF-LH	0.314	0.732	8.740	0.004	0.577	0.565	1.000	1.000	1.000	1.032	0.370	0.571	0.783	1.000	0.020	0.981	1.000	1.000	1.000
		LF-RH	1.434	0.246	0.001	0.978	4.087	0.022	0.198	0.976	1.000	0.444	0.646	1.000	1.000	1.000	6.168	0.005	0.005	0.616	0.079
		LH-RF	0.459	0.634	0.758	0.387	0.395	0.676	1.000	1.000	1.000	0.344	0.712	1.000	1.000	1.000	0.031	0.970	1.000	1.000	1.000
		RH-LF	1.701	0.191	0.017	0.896	3.368	0.041	0.149	1.000	0.773	0.293	0.748	1.000	1.000	1.000	5.977	0.006	0.007	1.000	0.051
		LH-RH	0.248	0.781	9.669	0.003	3.065	0.054	1.000	1.000	1.000	0.734	0.489	0.750	1.000	1.000	2.749	0.078	0.104	1.000	0.279
		LF-RF	0.421	0.658	1.210	0.276	1.902	0.158	0.890	1.000	1.000	0.327	0.724	1.000	1.000	1.000	1.867	0.170	0.186	0.941	0.983
		RH-LH	0.138	0.871	7.398	0.008	2.160	0.124	1.000	1.000	1.000	0.559	0.578	0.921	1.000	1.000	1.844	0.173	0.214	1.000	0.629
		RF-LF	0.201	0.818	0.838	0.364	1.727	0.186	1.000	1.000	1.000	0.532	0.593	1.000	1.000	1.000	1.364	0.269	0.328	0.839	1.000
		RF-RH	1.023	0.366	0.366	0.547	1.956	0.150	0.419	1.000	1.000	0.680	0.515	1.000	1.000	0.901	2.286	0.117	0.171	1.000	0.330
		LF-LH	0.803	0.452	9.812	0.003	0.098	0.907	0.658	1.000	1.000	0.341	0.714	1.000	1.000	1.000	0.595	0.557	0.914	1.000	1.000
		RH-RF	0.713	0.494	1.219	0.274	1.778	0.177	0.641	1.000	1.000	0.694	0.508	1.000	0.994	0.883	1.780	0.184	0.333	1.000	0.367
		LH-LF	1.288	0.283	11.260	0.001	0.066	0.937	0.362	1.000	1.000	0.497	0.614	1.000	1.000	1.000	0.917	0.409	0.566	1.000	1.000
individual paw	25	% Max contact at [%] RF	0.523	0.596	1.221	0.273	0.246	0.783	1.000	0.969	1.000	0.508	0.608	1.000	1.000	1.000	0.094	0.911	1.000	1.000	1.000
		RH	0.088	0.915	0.210	0.648	0.606	0.549	1.000	1.000	1.000	0.496	0.614	1.000	1.000	1.000	0.233	0.793	1.000	1.000	1.000
		LF	0.732	0.485	0.569	0.454	3.246	0.046	0.668	0.561	1.000	0.340	0.715	1.000	1.000	1.000	4.774	0.015	0.023	0.035	1.000
		LH	0.084	0.920	1.894	0.174	0.795	0.456	1.000	1.000	1.000	0.514	0.604	1.000	1.000	1.000	0.266	0.768	1.000	1.000	1.000
		FP	0.876	0.421	1.441	0.235	1.272	0.288	0.886	0.436	1.000	0.202	0.818	1.000	1.000	1.000	2.214	0.124	0.191	0.230	1.000
		HP	0.082	0.922	1.168	0.284	0.855	0.430	1.000	1.000	1.000	0.670	0.520	1.000	1.000	0.848	0.282	0.756	1.000	1.000	1.000
		RF	0.077	0.926	31.012	0.000	1.262	0.290	1.000	1.000	1.000	0.729	0.492	1.000	0.771	1.000	0.492	0.616	1.000	0.992	1.000
		RH	0.656	0.523	10.595	0.002	1.123	0.332	1.000	1.000	0.849	0.780	0.468	1.000	0.962	0.769	0.888	0.420	0.653	0.842	1.000
		LF	0.457	0.636	31.774	0.000	0.254	0.776	1.000	1.000	1.000	0.504	0.610	1.000	1.000	1.000	0.226	0.799	1.000	1.000	1.000
		LH	0.045	0.956	19.575	0.000	0.609	0.547	1.000	1.000	1.000	0.323	0.727	1.000	1.000	1.000	0.297	0.745	1.000	1.000	1.000
		FP	0.220	0.803	34.966	0.000	0.792	0.457	1.000	1.000	1.000	0.674	0.518	0.979	0.932	1.000	0.263	0.770	1.000	1.000	1.000
		HP	0.322	0.726	16.472	0.000	1.001	0.373	1.000	1.000	1.000	0.635	0.538	1.000	1.000	0.969	0.588	0.561	1.000	0.965	1.000
	26	Max contact max intensity [pixel] RF	0.074	0.929	8.376	0.005	1.524	0.226	1.000	1.000	1.000	0.798	0.461	1.000	0.698	1.000	0.718	0.495	1.000	0.721	1.000
		RH	0.224	0.800	8.360	0.005	0.378	0.686	1.000	1.000	1.000	0.174	0.841	1.000	1.000	1.000	0.411	0.666	1.000	1.000	1.000
		LF	0.458	0.635	6.101	0.016	0.756	0.474	1.000	1.000	1.000	0.620	0.545	1.000	0.934	1.000	0.453	0.640	1.000	1.000	1.000
		LH	0.132	0.877	10.898	0.002	1.049	0.357	1.000	1.000	1.000	1.135	0.336	1.000	0.566	0.625	0.299	0.743	1.000	1.000	1.000
		FP	0.240	0.788	7.692	0.007	1.167	0.318	1.000	1.000	1.000	0.732	0.490	1.000	0.787	1.000	0.534	0.591	1.000	1.000	1.000
		HP	0.166	0.848	10.631	0.002	0.730	0.486	1.000	1.000	1.000	0.587	0.563	1.000	1.000	1.000	0.375	0.690	1.000	1.000	1.000
	27	Max contact mean intensity [pixel] RF	0.300	0.742	2.408	0.126	0.937	0.397	1.000	1.000	1.000	0.675	0.518	1.000	0.788	1.000	0.367	0.695	1.000	1.000	1.000
		RH	0.252	0.778	3.347	0.072	0.369	0.693	1.000	1.000	1.000	0.272	0.764	1.000	1.000	1.000	0.321	0.728	1.000	1.000	1.000
		LF	0.300	0.742	6.032	0.017	0.633	0.535	1.000	1.000	1.000	0.495	0.615	1.000	0.994	1.000	0.202	0.818	1.000	1.000	1.000
		LH	0.827	0.442	4.950	0.030	1.671	0.196	1.000	1.000	0.736	1.701	0.202	1.000	0.448	0.291	0.426	0.657	1.000	1.000	1.000
		FP	0.320	0.728	4.214	0.044	0.818	0.446	1.000	1.000	1.000	0.608	0.551	1.000	0.854	1.000	0.305	0.739	1.000	1.000	1.000
		HP	0.519	0.598	4.887	0.031	0.965	0.387	1.000	1.000	1.000	0.926	0.408	1.000	0.847	0.648	0.403	0.671	1.000	1.000	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A34: ANOVA table (4) of CW testing of young Pink1 x Pet mice: individual paw

			Pink1 x Pet, young, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
individual paw	28 Maximum intensity [pixel]	RF	0.246	0.783	6.955	0.011	1.527	0.225	1.000	1.000	1.000	1.148	0.332	0.809	0.469	1.000	0.449	0.642	1.000	1.000	1.000
		RH	0.125	0.882	8.105	0.006	0.309	0.735	1.000	1.000	1.000	0.223	0.802	1.000	1.000	1.000	0.215	0.808	1.000	1.000	1.000
		LF	0.486	0.617	6.319	0.015	0.859	0.428	1.000	1.000	1.000	0.654	0.528	1.000	0.949	1.000	0.638	0.534	1.000	1.000	0.842
		LH	0.121	0.886	11.390	0.001	1.063	0.352	1.000	1.000	1.000	1.287	0.292	1.000	0.425	0.658	0.251	0.779	1.000	1.000	1.000
		FP	0.359	0.700	7.071	0.010	1.237	0.297	1.000	1.000	1.000	0.903	0.417	0.871	0.662	1.000	0.535	0.590	1.000	1.000	1.000
		HP	0.117	0.890	10.716	0.002	0.702	0.500	1.000	1.000	1.000	0.674	0.518	1.000	0.887	1.000	0.254	0.777	1.000	1.000	1.000
	29 % Maximum intensity at [sec]	RF	1.457	0.241	3.999	0.050	0.708	0.496	0.299	1.000	0.619	0.447	0.644	1.000	1.000	1.000	2.020	0.148	0.456	1.000	0.200
		RH	5.023	0.010	0.703	0.405	4.398	0.016	0.012	0.026	1.000	2.323	0.117	1.000	0.178	0.252	7.241	0.002	0.002	0.169	0.162
		LF	2.330	0.106	6.458	0.014	2.919	0.061	0.365	1.000	0.129	0.625	0.543	1.000	0.823	1.000	5.462	0.009	0.018	1.000	0.032
		LH	1.035	0.361	0.343	0.560	0.417	0.661	0.508	1.000	1.000	0.996	0.382	0.512	1.000	1.000	0.142	0.868	1.000	1.000	1.000
		FP	2.938	0.060	8.910	0.004	1.913	0.156	0.104	1.000	0.105	0.043	0.958	1.000	1.000	1.000	6.208	0.005	0.019	1.000	0.011
		HP	3.858	0.026	0.910	0.344	1.173	0.316	0.025	0.117	1.000	1.450	0.252	0.794	0.314	1.000	3.970	0.028	0.026	0.625	0.325
	30 Minimum intensity [pixel]	RF	0.027	0.973	6.093	0.016	1.528	0.225	1.000	1.000	1.000	1.042	0.366	0.541	0.833	1.000	0.582	0.564	0.886	1.000	1.000
		RH	0.082	0.921	6.263	0.015	1.319	0.275	1.000	1.000	1.000	1.288	0.292	0.522	0.506	1.000	0.505	0.608	0.967	1.000	1.000
		LF	1.277	0.286	0.064	0.802	3.142	0.050	0.448	1.000	1.000	4.455	0.021	0.062	0.032	1.000	0.595	0.557	1.000	1.000	1.000
		LH	0.091	0.913	9.296	0.003	0.373	0.690	1.000	1.000	1.000	0.158	0.855	1.000	1.000	1.000	0.320	0.728	1.000	1.000	1.000
		FP	0.452	0.639	2.130	0.150	2.572	0.085	1.000	1.000	1.000	2.774	0.080	0.139	0.155	1.000	0.465	0.632	1.000	1.000	1.000
		HP	0.025	0.976	9.485	0.003	0.792	0.457	1.000	1.000	1.000	1.111	0.344	0.759	0.513	1.000	0.221	0.802	1.000	1.000	1.000
	31 Mean intensity mean [pixel]	RF	0.399	0.673	1.545	0.219	1.003	0.373	1.000	1.000	1.000	0.893	0.421	1.000	0.592	1.000	0.173	0.842	1.000	1.000	1.000
		RH	0.175	0.840	3.015	0.087	0.405	0.669	1.000	1.000	1.000	0.252	0.779	1.000	1.000	1.000	0.299	0.743	1.000	1.000	1.000
		LF	0.369	0.693	5.647	0.021	0.748	0.478	1.000	1.000	1.000	0.593	0.559	1.000	0.868	1.000	0.308	0.737	1.000	1.000	1.000
		LH	0.480	0.621	4.999	0.029	1.547	0.221	1.000	1.000	1.000	1.304	0.288	1.000	0.525	0.488	0.353	0.705	1.000	1.000	1.000
		FP	0.401	0.671	3.361	0.072	0.891	0.416	1.000	1.000	1.000	0.762	0.477	1.000	0.697	1.000	0.239	0.789	1.000	1.000	1.000
		HP	0.316	0.730	4.502	0.038	0.921	0.403	1.000	1.000	1.000	0.762	0.477	1.000	0.852	0.883	0.308	0.737	1.000	1.000	1.000
	32 Intensity of 15 most intense pixel [pixel]	RF	0.295	0.745	7.395	0.008	1.196	0.309	1.000	1.000	1.000	0.849	0.439	1.000	0.625	1.000	0.302	0.741	1.000	1.000	1.000
		RH	0.239	0.788	9.867	0.003	0.631	0.535	1.000	1.000	1.000	0.409	0.668	1.000	1.000	1.000	0.414	0.664	1.000	1.000	1.000
		LF	0.466	0.630	10.543	0.002	0.758	0.473	1.000	1.000	1.000	0.648	0.531	1.000	0.877	1.000	0.370	0.693	1.000	1.000	1.000
		LH	0.259	0.773	12.603	0.001	1.384	0.258	1.000	1.000	1.000	1.118	0.342	1.000	0.569	0.645	0.388	0.681	1.000	1.000	1.000
		FP	0.374	0.689	9.118	0.004	1.005	0.372	1.000	1.000	1.000	0.770	0.473	1.000	0.716	1.000	0.317	0.730	1.000	1.000	1.000
		HP	0.258	0.774	12.035	0.001	1.033	0.362	1.000	1.000	1.000	0.782	0.468	1.000	0.811	0.892	0.408	0.668	1.000	1.000	1.000
	33 Print length [cm]	RF	0.045	0.956	7.538	0.008	0.476	0.624	1.000	1.000	1.000	0.307	0.738	1.000	1.000	1.000	0.158	0.854	1.000	1.000	1.000
		RH	0.148	0.863	12.651	0.001	0.445	0.643	1.000	1.000	1.000	0.231	0.795	1.000	1.000	1.000	0.358	0.702	1.000	1.000	1.000
		LF	0.183	0.834	19.727	0.000	0.053	0.949	1.000	1.000	1.000	0.024	0.976	1.000	1.000	1.000	0.254	0.777	1.000	1.000	1.000
		LH	0.259	0.773	31.789	0.000	0.287	0.751	1.000	1.000	1.000	0.518	0.601	1.000	1.000	0.959	0.040	0.961	1.000	1.000	1.000
		FP	0.119	0.888	13.868	0.000	0.125	0.882	1.000	1.000	1.000	0.180	0.836	1.000	1.000	1.000	0.033	0.967	1.000	1.000	1.000
		HP	0.247	0.782	22.342	0.000	0.390	0.678	1.000	1.000	1.000	0.411	0.667	1.000	1.000	1.000	0.241	0.787	1.000	1.000	1.000
	34 Print Width [cm]	RF	0.646	0.527	52.896	0.000	2.046	0.138	0.898	1.000	1.000	0.172	0.843	1.000	1.000	1.000	2.885	0.069	0.117	0.121	1.000
		RH	0.601	0.551	8.231	0.006	0.740	0.481	1.000	1.000	0.955	1.526	0.235	1.000	0.333	0.544	0.047	0.954	1.000	1.000	1.000
		LF	0.249	0.780	26.966	0.000	0.599	0.552	1.000	1.000	1.000	0.714	0.499	0.729	1.000	1.000	0.145	0.866	1.000	1.000	1.000
		LH	0.085	0.918	18.969	0.000	0.423	0.657	1.000	1.000	1.000	0.526	0.597	1.000	0.945	1.000	0.064	0.938	1.000	1.000	1.000
		FP	0.063	0.939	51.984	0.000	1.597	0.211	1.000	1.000	1.000	0.503	0.610	0.989	1.000	1.000	1.196	0.315	0.426	0.746	1.000
		HP	0.373	0.690	14.554	0.000	0.733	0.484	1.000	1.000	1.000	1.312	0.286	1.000	0.381	0.765	0.059	0.943	1.000	1.000	1.000
	35 Print area [cm]	RF	0.027	0.973	23.870	0.000	1.353	0.266	1.000	1.000	1.000	0.694	0.508	1.000	0.793	1.000	0.632	0.537	1.000	0.827	1.000
		RH	0.644	0.529	12.293	0.001	0.976	0.383	1.000	1.000	0.795	0.768	0.474	1.000	0.974	0.779	0.728	0.490	0.763	1.000	1.000
		LF	0.608	0.548	28.096	0.000	0.692	0.504	0.815	1.000	1.000	1.074	0.356	0.556	0.734	1.000	0.214	0.809	1.000	1.000	1.000
		LH	0.115	0.892	24.830	0.000	0.608	0.547	1.000	1.000	1.000	0.459	0.637	1.000	1.000	1.000	0.182	0.834	1.000	1.000	1.000
		FP	0.201	0.818	28.098	0.000	1.163	0.319	1.000	1.000	1.000	0.947	0.400	0.768	0.671	1.000	0.333	0.719	1.000	1.000	1.000
		HP	0.408	0.667	20.050	0.000	0.940	0.396	1.000	1.000	0.982	0.743	0.485	1.000	0.933	0.844	0.464	0.632	1.000	1.000	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A35: ANOVA table (1) of CW testing of mid-aged Pink1 x Pet mice: temporal parameters

			Pink1 x Pet, mid-aged, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
temporal parameters	1	Cadence [steps/sec]	0.415	0.662	4.523	0.037	2.305	0.108	1.000	1.000	1.000	2.550	0.095	0.619	0.986	0.100	0.788	0.463	1.000	0.785	0.997
	2	Stance duration [sec] RF	1.436	0.246	5.998	0.017	1.918	0.156	0.322	1.000	1.000	3.663	0.038	0.518	0.563	0.034	0.970	0.390	0.866	0.577	1.000
		RH	0.996	0.375	2.993	0.089	2.306	0.108	0.697	1.000	1.000	3.635	0.039	0.654	0.452	0.035	0.582	0.564	1.000	0.866	1.000
		LF	0.108	0.898	7.451	0.008	1.393	0.256	1.000	1.000	1.000	1.901	0.168	1.000	0.682	0.186	0.333	0.719	1.000	1.000	1.000
		LH	0.438	0.647	5.280	0.025	2.492	0.091	1.000	0.696	1.000	2.232	0.125	0.362	1.000	0.181	1.255	0.299	1.000	0.610	0.513
		FP	0.609	0.547	7.124	0.010	1.721	0.187	0.896	1.000	1.000	2.971	0.067	0.843	0.543	0.063	0.547	0.584	1.000	0.910	1.000
		HP	0.666	0.518	5.087	0.028	2.887	0.063	0.740	1.000	1.000	4.129	0.026	0.269	0.792	0.025	0.951	0.397	1.000	0.624	0.928
	3	Stand [sec] RF	0.503	0.607	2.402	0.126	2.437	0.096	1.000	0.583	1.000	2.027	0.150	0.487	1.000	0.198	1.475	0.244	1.000	0.451	0.463
		RH	0.593	0.556	0.612	0.437	1.459	0.241	1.000	0.582	1.000	0.646	0.532	1.000	1.000	0.917	1.363	0.270	1.000	0.423	0.624
		LF	0.229	0.796	2.997	0.088	2.431	0.096	1.000	0.966	1.000	1.541	0.231	0.540	1.000	0.363	1.427	0.255	1.000	1.000	0.311
		LH	1.398	0.255	1.190	0.280	1.768	0.179	1.000	0.165	0.627	0.754	0.479	0.716	1.000	1.000	2.230	0.124	1.000	0.235	0.250
		FP	0.326	0.723	2.718	0.104	2.420	0.097	1.000	0.745	1.000	1.826	0.179	0.491	1.000	0.258	1.403	0.261	1.000	0.684	0.376
		HP	0.991	0.377	0.897	0.347	1.676	0.196	1.000	0.295	0.978	0.709	0.500	0.869	1.000	1.000	1.829	0.177	1.000	0.302	0.381
	4	Stand index [cm/sec] RF	0.890	0.416	0.570	0.453	2.140	0.126	0.615	1.000	1.000	2.810	0.077	0.951	0.535	0.074	1.205	0.313	0.987	0.397	1.000
		RH	0.571	0.568	1.283	0.262	0.268	0.766	1.000	1.000	1.000	0.868	0.431	0.629	1.000	1.000	0.150	0.861	1.000	1.000	1.000
		LF	0.603	0.550	2.098	0.153	0.899	0.412	0.903	1.000	1.000	1.071	0.356	1.000	1.000	0.479	0.667	0.520	1.000	0.784	1.000
		LH	0.197	0.822	2.019	0.160	0.633	0.534	1.000	1.000	1.000	0.265	0.769	1.000	1.000	1.000	0.521	0.599	1.000	1.000	1.000
		FP	0.835	0.439	1.407	0.240	1.589	0.212	0.663	1.000	1.000	2.133	0.137	0.894	0.924	0.145	1.003	0.378	1.000	0.508	1.000
		HP	0.020	0.980	2.112	0.151	0.510	0.603	1.000	1.000	1.000	0.285	0.754	1.000	1.000	1.000	0.269	0.766	1.000	1.000	1.000
	5	Body speed [cm/sec] RF	0.675	0.513	1.185	0.281	1.043	0.358	0.750	1.000	1.000	1.880	0.171	0.413	1.000	0.268	0.305	0.739	1.000	1.000	1.000
		RH	0.617	0.543	0.750	0.390	1.120	0.333	0.863	1.000	1.000	2.099	0.141	0.387	1.000	0.207	0.217	0.806	1.000	1.000	1.000
		LF	0.690	0.505	1.594	0.212	1.114	0.335	0.762	1.000	1.000	2.049	0.147	0.378	1.000	0.225	0.236	0.791	1.000	1.000	1.000
		LH	0.725	0.488	1.203	0.277	0.969	0.385	0.725	1.000	1.000	1.846	0.176	0.445	1.000	0.267	0.261	0.772	1.000	1.000	1.000
		FP	0.683	0.509	1.387	0.244	1.077	0.347	0.754	1.000	1.000	1.969	0.158	0.394	1.000	0.245	0.270	0.765	1.000	1.000	1.000
		HP	0.678	0.511	0.970	0.329	1.052	0.355	0.781	1.000	1.000	2.001	0.153	0.404	1.000	0.230	0.239	0.789	1.000	1.000	1.000
	6	Body speed variation [%] RF	0.492	0.614	0.720	0.399	0.158	0.854	1.000	1.000	0.803	0.038	0.963	1.000	1.000	1.000	0.824	0.448	1.000	1.000	0.626
		RH	0.223	0.801	3.335	0.073	0.098	0.907	1.000	1.000	1.000	0.227	0.798	1.000	1.000	1.000	0.027	0.974	1.000	1.000	1.000
		LF	0.120	0.887	0.151	0.699	0.395	0.675	1.000	1.000	1.000	0.294	0.748	1.000	1.000	1.000	0.253	0.778	1.000	1.000	1.000
		LH	0.426	0.655	2.848	0.097	0.462	0.632	1.000	1.000	0.869	0.091	0.914	1.000	1.000	1.000	0.912	0.412	1.000	1.000	0.560
		FP	0.301	0.741	0.446	0.507	0.193	0.825	1.000	1.000	1.000	0.115	0.891	1.000	1.000	1.000	0.459	0.636	1.000	1.000	1.000
		HP	0.201	0.819	3.497	0.066	0.035	0.965	1.000	1.000	1.000	0.027	0.973	1.000	1.000	1.000	0.262	0.771	1.000	1.000	1.000
	7	Number of steps [#]	0.512	0.602	0.339	0.563	0.881	0.419	1.000	1.000	0.855	0.778	0.469	0.763	1.000	1.000	0.667	0.520	1.000	0.772	1.000
	8	Swing duration [sec] RF	0.161	0.852	10.947	0.002	1.223	0.302	1.000	1.000	1.000	1.402	0.262	1.000	0.906	0.318	0.305	0.739	1.000	1.000	1.000
		RH	0.335	0.717	12.457	0.001	2.266	0.112	1.000	1.000	1.000	2.784	0.078	0.784	0.665	0.076	0.462	0.634	1.000	1.000	1.000
		LF	2.008	0.143	9.358	0.003	1.665	0.198	0.179	1.000	0.884	3.085	0.061	0.498	0.850	0.060	1.146	0.331	0.579	0.569	1.000
		LH	1.726	0.187	10.183	0.002	1.669	0.197	1.000	1.000	0.469	4.230	0.024	1.000	0.172	0.023	0.136	0.874	1.000	1.000	1.000
		FP	0.878	0.421	10.977	0.002	1.526	0.226	0.642	1.000	1.000	2.331	0.115	0.895	0.800	0.118	0.595	0.558	1.000	0.873	1.000
		HP	1.022	0.366	13.356	0.001	2.292	0.110	1.000	1.000	1.000	4.409	0.021	0.745	0.240	0.018	0.209	0.813	1.000	1.000	1.000
	9	Swing speed [cm/sec] RF	0.753	0.475	7.245	0.009	1.005	0.372	1.000	1.000	1.000	1.950	0.160	0.686	1.000	0.188	0.014	0.987	1.000	1.000	1.000
		RH	0.752	0.476	10.472	0.002	2.572	0.085	1.000	1.000	1.000	3.039	0.063	0.415	1.000	0.066	0.279	0.759	1.000	1.000	1.000
		LF	1.765	0.180	7.571	0.008	1.435	0.246	0.304	1.000	0.686	3.083	0.061	0.444	0.948	0.062	0.540	0.588	1.000	1.000	1.000
		LH	2.822	0.067	10.216	0.002	1.717	0.188	0.794	1.000	0.163	3.857	0.033	0.840	0.301	0.029	0.120	0.887	1.000	1.000	1.000
		FP	1.209	0.305	7.740	0.007	1.199	0.309	0.628	1.000	0.871	2.582	0.093	0.540	1.000	0.100	0.171	0.844	1.000	1.000	1.000
		HP	1.930	0.154	12.369	0.001	2.459	0.094	0.908	1.000	0.412	4.213	0.025	0.468	0.441	0.021	0.020	0.980	1.000	1.000	1.000
	10	Run duration [sec]	0.036	0.965	1.833	0.181	1.761	0.181	1.000	1.000	1.000	1.532	0.233	0.639	1.000	0.331	0.669	0.519	1.000	1.000	0.769
	11	Run speed [cm/sec]	0.389	0.680	2.336	0.132	1.191	0.311	1.000	1.000	1.000	1.352	0.275	0.797	1.000	0.379	0.438	0.649	1.000	1.000	1.000
	12	Run maximum variation [%]	1.955	0.150	2.740	0.103	0.203	0.817	0.172	1.000	0.583	1.205	0.314	0.420	0.945	1.000	1.057	0.359	0.568	1.000	0.785

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A36: ANOVA table (2) of CW testing of mid-aged Pink1 x Pet mice: comparative paws

			Pink1 x Pet, mid-aged, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
comparative paws	13 Stride length [cm]	RF	0.253	0.777	0.000	0.986	0.851	0.432	1.000	1.000	1.000	0.876	0.427	0.621	1.000	1.000	0.209	0.813	1.000	1.000	1.000
		RH	0.225	0.799	0.005	0.943	0.986	0.379	1.000	1.000	1.000	0.839	0.443	0.652	1.000	1.000	0.359	0.701	1.000	1.000	1.000
		LF	0.068	0.934	0.011	0.918	0.607	0.548	1.000	1.000	1.000	0.499	0.613	1.000	1.000	1.000	0.154	0.858	1.000	1.000	1.000
		LH	0.166	0.847	0.000	0.994	0.448	0.641	1.000	1.000	1.000	0.434	0.652	1.000	1.000	1.000	0.160	0.852	1.000	1.000	1.000
		FP	0.146	0.865	0.002	0.964	0.721	0.491	1.000	1.000	1.000	0.675	0.517	0.813	1.000	1.000	0.165	0.849	1.000	1.000	1.000
		HP	0.195	0.823	0.001	0.977	0.687	0.507	1.000	1.000	1.000	0.617	0.547	0.890	1.000	1.000	0.247	0.783	1.000	1.000	1.000
	14 Step cycle [sec]	RF	0.348	0.707	5.738	0.020	2.393	0.100	1.000	0.756	1.000	2.475	0.102	0.561	1.000	0.111	1.199	0.315	1.000	0.552	0.627
		RH	0.439	0.647	5.044	0.028	2.660	0.078	1.000	0.717	1.000	2.640	0.088	0.476	1.000	0.097	1.323	0.280	1.000	0.473	0.586
		LF	0.350	0.706	5.954	0.018	2.167	0.123	1.000	0.997	1.000	2.899	0.071	0.330	1.000	0.084	0.835	0.443	1.000	0.837	0.833
		LH	0.532	0.590	6.107	0.016	2.585	0.084	0.932	0.694	1.000	2.857	0.074	0.372	1.000	0.084	1.231	0.305	1.000	0.492	0.675
		FP	0.342	0.712	5.865	0.018	2.274	0.112	1.000	0.869	1.000	2.695	0.084	0.429	1.000	0.095	1.011	0.375	1.000	0.680	0.724
		HP	0.482	0.620	5.583	0.021	2.635	0.080	1.000	0.706	1.000	2.772	0.079	0.418	1.000	0.088	1.279	0.292	1.000	0.482	0.627
	15 Duty cycle [%]	RF	1.051	0.356	1.022	0.316	0.790	0.459	1.000	0.432	1.000	0.451	0.642	1.000	1.000	1.000	1.396	0.262	1.000	0.394	0.644
		RH	0.709	0.496	3.995	0.050	0.103	0.902	1.000	0.965	1.000	0.286	0.753	1.000	1.000	1.000	0.513	0.604	1.000	0.976	1.000
		LF	1.277	0.286	0.568	0.454	1.514	0.228	0.897	1.000	0.372	0.216	0.807	1.000	1.000	1.000	2.399	0.107	0.246	1.000	0.173
		LH	2.883	0.064	2.156	0.147	0.161	0.852	1.000	0.131	0.182	1.749	0.192	1.000	0.226	0.626	1.439	0.252	1.000	0.632	0.374
		FP	0.804	0.452	0.938	0.337	0.990	0.377	1.000	0.869	0.718	0.327	0.724	1.000	1.000	1.000	1.472	0.245	1.000	1.000	0.290
		HP	2.013	0.142	4.110	0.047	0.004	0.996	1.000	0.274	0.503	1.161	0.327	1.000	0.457	0.771	0.916	0.410	1.000	0.675	0.900
	16 Base of support [cm]	FP	2.130	0.128	0.157	0.693	0.975	0.383	0.655	0.105	1.000	0.141	0.869	1.000	1.000	1.000	3.218	0.053	0.286	0.052	1.000
		HP	0.623	0.539	1.032	0.314	2.107	0.130	1.000	1.000	1.000	1.034	0.368	1.000	0.493	1.000	1.514	0.235	0.310	0.592	1.000
	17 Initial dual stance [sec]	RF	1.337	0.270	0.231	0.633	1.237	0.298	1.000	0.247	0.475	0.120	0.888	1.000	1.000	1.000	2.801	0.076	1.000	0.158	0.153
		RH	2.364	0.103	0.595	0.444	0.198	0.821	1.000	0.136	0.432	0.810	0.455	1.000	0.814	0.807	1.981	0.154	0.956	0.166	0.967
		LF	0.514	0.601	0.000	0.993	3.713	0.030	1.000	1.000	0.775	0.959	0.395	0.696	1.000	0.797	3.018	0.063	0.217	1.000	0.083
		LH	1.087	0.344	0.025	0.876	1.141	0.326	1.000	0.800	0.367	0.132	0.877	1.000	1.000	1.000	1.832	0.177	1.000	1.000	0.195
		FP	0.780	0.463	0.081	0.777	2.426	0.097	1.000	0.740	0.506	0.493	0.616	1.000	1.000	1.000	2.689	0.083	0.932	0.769	0.081
		HP	2.119	0.129	0.264	0.610	0.284	0.753	1.000	0.204	0.224	0.468	0.631	1.000	1.000	1.000	2.032	0.148	1.000	0.331	0.248
	18 Terminal dual stance [sec]	RF	0.792	0.457	0.017	0.896	3.551	0.035	1.000	1.000	0.475	0.737	0.487	0.789	1.000	1.000	3.319	0.049	0.225	1.000	0.057
		RH	1.278	0.286	0.009	0.923	1.119	0.333	1.000	0.618	0.283	0.025	0.976	1.000	1.000	1.000	2.106	0.138	1.000	0.593	0.161
		LF	1.218	0.303	0.146	0.704	0.864	0.426	1.000	0.277	0.722	0.234	0.793	1.000	1.000	1.000	2.264	0.120	1.000	0.227	0.246
		LH	2.566	0.085	0.410	0.524	0.163	0.850	1.000	0.117	0.330	0.999	0.381	1.000	0.673	0.659	1.840	0.175	1.000	0.197	0.882
		FP	0.965	0.387	0.071	0.791	2.254	0.114	1.000	0.551	0.446	0.541	0.588	0.930	1.000	1.000	2.716	0.081	0.986	0.713	0.080
		HP	2.507	0.090	0.211	0.647	0.284	0.754	1.000	0.143	0.157	0.541	0.588	1.000	1.000	1.000	2.363	0.110	1.000	0.223	0.214
	19 Print position right paws [cm]		0.079	0.924	6.700	0.012	5.529	0.006	1.000	1.000	1.000	2.697	0.084	0.083	0.681	1.000	2.922	0.068	0.081	0.219	1.000
	20 Print position left paws [cm]		0.317	0.730	3.143	0.081	2.122	0.129	1.000	1.000	1.000	0.719	0.496	1.000	1.000	0.850	1.661	0.206	0.237	1.000	0.930

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A37: ANOVA table (3) of CW testing of mid-aged Pink1 x Pet mice: interlimb coordination and individual paw

				Pink1 x Pet, mid-aged, CW										males					females				
				Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
				F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
interlimb coordination	21	Number of patterns		0.769	0.468	0.027	0.869	0.961	0.388	1.000	1.000	0.517	0.596	0.558	0.854	1.000	1.000	1.290	0.289	1.000	0.523	0.558
	22	Regularity index [%]		0.735	0.484	0.253	0.617	1.241	0.296	1.000	1.000	0.977	1.366	0.271	1.000	0.770	0.344	0.526	0.596	1.000	1.000	1.000
	23	Phase dispersion [%]	RF-LH	1.146	0.325	0.577	0.450	0.833	0.440	0.565	1.000	0.718	0.859	0.434	1.000	1.000	0.699	1.662	0.206	0.400	0.316	1.000
			LF-RH	0.729	0.487	0.730	0.396	2.221	0.117	0.764	1.000	1.000	0.694	0.508	1.000	1.000	0.804	2.233	0.124	0.210	1.000	0.262
			LH-RH	0.287	0.751	0.055	0.816	0.734	0.484	1.000	1.000	1.000	0.050	0.951	1.000	1.000	1.000	1.183	0.319	0.477	1.000	0.755
			LF-RF	3.277	0.044	0.309	0.580	5.500	0.006	0.097	0.077	1.000	0.215	0.808	1.000	1.000	1.000	8.358	0.001	0.002	0.005	1.000
			RF-RH	0.048	0.953	1.357	0.249	2.066	0.136	1.000	1.000	1.000	0.570	0.572	1.000	1.000	1.000	1.832	0.177	1.000	0.288	0.401
			LF-LH	0.049	0.952	0.143	0.707	0.378	0.687	1.000	1.000	1.000	0.110	0.896	1.000	1.000	1.000	0.437	0.650	1.000	1.000	1.000
	24	Couplings [%]	RF-LH	1.872	0.163	3.052	0.086	2.686	0.076	0.370	1.000	0.228	2.173	0.132	0.217	1.000	0.314	1.365	0.270	0.436	0.497	1.000
			LF-RH	0.801	0.454	0.661	0.419	1.177	0.315	0.986	0.726	1.000	0.699	0.505	1.000	1.000	0.789	1.082	0.351	0.941	0.473	1.000
			LH-RF	1.867	0.163	1.516	0.223	1.203	0.307	1.000	0.358	0.652	1.310	0.285	1.000	0.502	0.479	1.555	0.227	0.383	0.396	1.000
			RH-LF	0.901	0.411	1.371	0.246	2.540	0.087	1.000	1.000	0.328	0.603	0.554	1.000	1.000	0.960	2.264	0.120	0.934	1.000	0.123
			LH-RH	0.371	0.692	0.066	0.798	0.642	0.530	1.000	1.000	1.000	0.029	0.971	1.000	1.000	1.000	1.288	0.290	0.473	1.000	0.598
			LF-RF	2.621	0.081	0.395	0.532	3.813	0.028	0.101	0.288	1.000	0.099	0.906	1.000	1.000	1.000	5.818	0.007	0.008	0.035	1.000
			RH-LH	0.392	0.677	0.128	0.721	0.684	0.509	1.000	1.000	1.000	0.105	0.900	1.000	1.000	1.000	1.157	0.327	0.446	1.000	0.937
			RF-LF	3.059	0.054	0.502	0.481	3.356	0.041	0.122	0.085	1.000	0.169	0.846	1.000	1.000	1.000	6.074	0.006	0.008	0.023	1.000
			RF-RH	0.054	0.947	1.376	0.245	2.041	0.139	1.000	1.000	1.000	0.535	0.592	1.000	1.000	1.000	1.913	0.164	1.000	0.285	0.350
			LF-LH	0.024	0.976	0.085	0.772	0.398	0.673	1.000	1.000	1.000	0.110	0.896	1.000	1.000	1.000	0.433	0.652	1.000	1.000	1.000
			RH-RF	0.023	0.977	1.140	0.290	1.921	0.155	1.000	1.000	1.000	0.651	0.529	1.000	1.000	0.885	1.449	0.250	1.000	0.449	0.489
			LH-LF	0.018	0.982	0.089	0.767	0.249	0.780	1.000	1.000	1.000	0.087	0.917	1.000	1.000	1.000	0.230	0.796	1.000	1.000	1.000
individual paw	25	% Max contact at [%]	RF	3.116	0.051	2.591	0.113	1.385	0.258	1.000	0.086	0.114	0.359	0.702	1.000	1.000	1.000	4.657	0.017	1.000	0.020	0.114
			RH	1.043	0.358	2.436	0.124	0.543	0.584	1.000	0.434	0.988	1.309	0.286	1.000	0.425	0.585	0.141	0.869	1.000	1.000	1.000
			LF	1.903	0.158	4.702	0.034	6.626	0.002	1.000	0.376	0.608	3.350	0.049	0.124	1.000	0.092	5.151	0.012	0.015	0.043	1.000
			LH	1.057	0.354	1.777	0.188	0.362	0.698	0.425	1.000	1.000	0.776	0.469	0.809	0.958	1.000	0.693	0.507	1.000	1.000	0.871
			FP	3.916	0.025	5.570	0.021	4.164	0.020	1.000	0.060	0.106	2.174	0.132	0.368	1.000	0.193	6.639	0.004	0.056	0.003	0.798
			HP	0.954	0.391	3.047	0.086	0.600	0.552	0.654	0.615	1.000	1.144	0.332	1.000	0.427	1.000	0.421	0.660	1.000	1.000	1.000
			RF	4.063	0.022	30.499	0.000	0.802	0.453	0.269	1.000	0.049	6.371	0.005	0.019	1.000	0.012	1.325	0.280	1.000	0.676	0.425
			RH	0.552	0.579	42.400	0.000	4.602	0.014	1.000	1.000	1.000	1.383	0.267	0.592	0.414	1.000	4.040	0.027	0.836	0.026	0.267
			LF	5.342	0.007	30.501	0.000	0.869	0.425	1.000	0.106	0.040	4.837	0.015	0.657	0.210	0.013	2.151	0.133	1.000	0.160	0.529
			LH	1.511	0.229	29.770	0.000	1.859	0.164	1.000	0.739	0.556	0.014	0.986	1.000	1.000	1.000	4.369	0.021	1.000	0.048	0.053
			FP	5.002	0.010	35.748	0.000	0.952	0.392	0.738	0.444	0.027	6.794	0.004	0.068	0.633	0.004	1.745	0.191	1.000	0.320	0.415
			HP	1.201	0.308	42.581	0.000	3.255	0.045	1.000	1.000	0.740	0.406	0.670	1.000	1.000	1.000	4.664	0.017	1.000	0.023	0.086
	26	Max contact max intensity [pixel]	RF	0.300	0.742	13.130	0.001	0.393	0.677	1.000	1.000	1.000	0.054	0.947	1.000	1.000	1.000	0.634	0.537	1.000	1.000	0.808
			RH	3.575	0.034	25.443	0.000	2.089	0.133	1.000	0.671	0.089	3.545	0.042	0.208	1.000	0.051	1.750	0.190	1.000	0.216	0.952
			LF	0.731	0.486	17.732	0.000	0.709	0.496	1.000	1.000	0.988	0.029	0.972	1.000	1.000	1.000	1.463	0.247	1.000	1.000	0.297
			LH	2.450	0.095	16.389	0.000	0.081	0.922	1.000	0.735	0.184	0.766	0.474	1.000	1.000	0.677	2.180	0.130	1.000	0.363	0.188
			FP	0.508	0.604	16.896	0.000	0.572	0.567	1.000	1.000	1.000	0.002	0.998	1.000	1.000	1.000	1.061	0.358	1.000	1.000	0.469
			HP	3.412	0.039	23.847	0.000	0.679	0.511	1.000	0.623	0.092	2.004	0.153	0.653	1.000	0.180	2.069	0.143	1.000	0.224	0.354
	27	Max contact mean intensity [pixel]	RF	1.379	0.259	13.563	0.000	0.631	0.536	0.731	1.000	0.436	0.363	0.699	1.000	1.000	1.000	1.742	0.191	1.000	0.908	0.219
			RH	4.357	0.017	7.339	0.009	1.651	0.200	0.327	1.000	0.033	4.737	0.017	0.201	0.726	0.016	0.461	0.635	1.000	1.000	1.000
			LF	2.055	0.137	24.345	0.000	0.319	0.728	1.000	1.000	0.254	0.513	0.604	1.000	1.000	1.000	1.861	0.172	1.000	0.668	0.208
			LH	1.157	0.321	9.951	0.002	0.001	0.999	1.000	1.000	0.631	0.396	0.677	1.000	1.000	1.000	0.893	0.420	1.000	1.000	0.616
			FP	1.759	0.181	19.513	0.000	0.487	0.617	0.813	1.000	0.307	0.412	0.666	1.000	1.000	1.000	1.868	0.171	1.000	0.766	0.197
			HP	3.012	0.057	10.319	0.002	0.484	0.619	0.706	1.000	0.104	2.357	0.113	0.698	1.000	0.119	0.761	0.475	1.000	1.000	0.718

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A38: ANOVA table (4) of CW testing of mid-aged Pink1 x Pet mice: individual paw

			Pink1 x Pet, mid-aged, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
individual paw	28 Maximum intensity [pixel]	RF	0.514	0.601	13.498	0.001	0.441	0.645	1.000	1.000	1.000	0.051	0.951	1.000	1.000	1.000	0.948	0.398	1.000	1.000	0.576
		RH	3.125	0.051	30.196	0.000	1.731	0.186	1.000	0.696	0.140	2.684	0.085	0.308	1.000	0.112	2.062	0.144	1.000	0.157	0.782
		LF	0.866	0.426	22.415	0.000	0.734	0.484	1.000	1.000	0.918	0.032	0.969	1.000	1.000	1.000	1.547	0.228	1.000	0.830	0.284
		LH	2.218	0.118	20.137	0.000	0.248	0.781	1.000	0.777	0.231	0.431	0.654	1.000	1.000	1.000	2.517	0.097	1.000	0.312	0.133
		FP	0.713	0.494	18.949	0.000	0.582	0.562	1.000	1.000	1.000	0.006	0.994	1.000	1.000	1.000	1.284	0.291	1.000	0.931	0.384
		HP	3.020	0.056	28.710	0.000	0.545	0.582	1.000	0.662	0.134	1.398	0.263	0.855	1.000	0.345	2.389	0.108	1.000	0.179	0.261
	29 % Maximum intensity at [sec]	RF	0.690	0.506	1.665	0.202	0.036	0.965	0.745	1.000	1.000	0.179	0.837	1.000	1.000	1.000	0.602	0.554	0.881	1.000	1.000
		RH	0.506	0.606	0.048	0.827	3.417	0.039	1.000	1.000	1.000	2.327	0.116	0.872	0.823	0.119	1.187	0.318	0.614	1.000	0.551
		LF	0.206	0.814	6.796	0.011	3.517	0.036	1.000	1.000	1.000	3.238	0.054	0.050	0.810	0.653	0.892	0.420	0.598	1.000	1.000
		LH	0.736	0.483	0.413	0.523	0.784	0.461	1.000	0.761	1.000	1.104	0.345	1.000	0.568	0.634	0.169	0.845	1.000	1.000	1.000
		FP	0.086	0.918	5.285	0.025	1.187	0.312	1.000	1.000	1.000	0.323	0.727	1.000	1.000	1.000	1.009	0.376	0.533	1.000	1.000
		HP	0.806	0.451	0.063	0.802	2.392	0.100	1.000	0.881	1.000	2.109	0.140	1.000	0.464	0.160	0.699	0.505	0.890	1.000	1.000
	30 Minimum intensity [pixel]	RF	0.869	0.424	4.601	0.036	0.843	0.436	1.000	0.811	0.878	0.101	0.905	1.000	1.000	1.000	1.159	0.327	1.000	0.487	0.817
		RH	1.286	0.284	20.941	0.000	3.030	0.056	0.813	0.669	1.000	0.496	0.614	1.000	1.000	1.000	2.802	0.076	0.358	0.077	1.000
		LF	0.056	0.945	0.369	0.546	0.294	0.746	1.000	1.000	1.000	0.137	0.872	1.000	1.000	1.000	0.223	0.802	1.000	1.000	1.000
		LH	2.381	0.101	8.390	0.005	0.269	0.765	1.000	0.198	0.381	0.607	0.552	1.000	0.907	1.000	1.986	0.154	1.000	0.221	0.425
		FP	0.492	0.614	2.858	0.096	0.771	0.467	1.000	1.000	1.000	0.143	0.867	1.000	1.000	1.000	0.865	0.431	1.000	0.703	0.962
		HP	1.897	0.159	19.276	0.000	1.813	0.172	1.000	0.296	1.000	0.005	0.995	1.000	1.000	1.000	2.751	0.079	0.659	0.076	0.807
	31 Mean intensity mean [pixel]	RF	1.089	0.343	8.795	0.004	0.724	0.489	1.000	1.000	0.523	0.258	0.774	1.000	1.000	1.000	1.510	0.236	1.000	0.909	0.289
		RH	4.506	0.015	8.321	0.005	0.731	0.486	0.344	0.828	0.023	3.301	0.051	0.280	1.000	0.058	1.502	0.238	1.000	0.676	0.329
		LF	1.741	0.184	22.118	0.000	0.395	0.675	1.000	1.000	0.339	0.343	0.713	1.000	1.000	1.000	1.743	0.191	1.000	0.643	0.245
		LH	1.346	0.268	10.738	0.002	0.457	0.635	1.000	1.000	0.433	0.147	0.864	1.000	1.000	1.000	2.151	0.133	1.000	0.387	0.188
		FP	1.428	0.248	15.205	0.000	0.575	0.566	1.000	1.000	0.398	0.267	0.768	1.000	1.000	1.000	1.666	0.205	1.000	0.763	0.252
		HP	3.068	0.054	10.963	0.002	0.236	0.790	0.729	0.896	0.079	1.450	0.251	0.750	1.000	0.341	2.005	0.151	1.000	0.465	0.204
	32 Intensity of 15 most intense pixel [pixel]	RF	1.243	0.296	15.082	0.000	0.397	0.674	0.858	1.000	0.533	0.460	0.636	1.000	1.000	1.000	1.218	0.309	1.000	1.000	0.401
		RH	2.927	0.061	24.866	0.000	1.290	0.283	0.994	0.910	0.130	2.337	0.115	0.306	1.000	0.176	1.900	0.166	1.000	0.216	0.543
		LF	1.450	0.243	26.360	0.000	0.401	0.671	1.000	1.000	0.501	0.192	0.826	1.000	1.000	1.000	1.574	0.223	1.000	0.790	0.279
		LH	1.881	0.161	19.070	0.000	0.463	0.632	1.000	0.843	0.305	0.236	0.791	1.000	1.000	1.000	2.666	0.085	1.000	0.253	0.125
		FP	1.368	0.262	21.261	0.000	0.390	0.679	1.000	1.000	0.487	0.289	0.751	1.000	1.000	1.000	1.435	0.253	1.000	0.910	0.318
		HP	2.660	0.078	24.971	0.000	0.568	0.570	1.000	0.807	0.158	1.085	0.351	0.877	1.000	0.529	2.431	0.104	1.000	0.197	0.218
	33 Print length [cm]	RF	4.184	0.020	2.604	0.112	1.421	0.249	0.244	0.934	0.018	3.673	0.038	0.061	1.000	0.125	2.381	0.109	1.000	0.267	0.178
		RH	0.831	0.440	43.287	0.000	4.426	0.016	0.895	1.000	1.000	2.106	0.140	1.000	0.262	0.222	2.985	0.065	0.463	0.061	0.971
		LF	1.255	0.292	1.042	0.311	0.263	0.769	1.000	0.584	0.752	1.893	0.169	1.000	0.210	0.459	0.184	0.833	1.000	1.000	1.000
		LH	0.956	0.390	24.532	0.000	3.732	0.030	0.725	1.000	1.000	1.507	0.238	0.787	1.000	0.310	3.904	0.030	1.000	0.040	0.143
		FP	2.638	0.080	2.243	0.139	0.178	0.837	1.000	0.585	0.100	2.225	0.126	0.760	1.000	0.135	1.000	0.379	1.000	0.822	0.625
		HP	1.118	0.334	42.151	0.000	4.672	0.013	0.652	1.000	1.000	1.909	0.166	1.000	0.754	0.182	3.919	0.030	0.720	0.028	0.339
	34 Print Width [cm]	RF	0.447	0.641	13.623	0.000	0.748	0.477	1.000	1.000	1.000	1.287	0.291	0.449	1.000	0.694	0.271	0.764	1.000	1.000	1.000
		RH	0.030	0.970	28.304	0.000	2.774	0.070	1.000	1.000	1.000	1.558	0.228	0.751	0.287	1.000	1.348	0.274	1.000	0.339	1.000
		LF	0.549	0.580	2.885	0.094	1.154	0.322	1.000	1.000	1.000	0.488	0.619	1.000	1.000	1.000	1.264	0.296	0.742	0.399	1.000
		LH	0.991	0.377	35.499	0.000	1.713	0.189	1.000	1.000	0.866	0.201	0.819	1.000	1.000	1.000	2.545	0.094	1.000	0.160	0.228
		FP	0.541	0.585	9.515	0.003	1.203	0.307	1.000	1.000	1.000	1.016	0.375	0.603	1.000	0.846	0.802	0.457	1.000	0.675	1.000
		HP	0.397	0.674	40.232	0.000	2.596	0.083	1.000	1.000	1.000	0.893	0.420	0.985	0.650	1.000	2.131	0.135	1.000	0.181	0.430
	35 Print area [cm]	RF	2.499	0.091	16.071	0.000	1.137	0.327	0.475	1.000	0.147	4.062	0.028	0.038	1.000	0.126	1.208	0.312	1.000	0.601	0.565
		RH	0.228	0.797	46.399	0.000	4.648	0.013	1.000	1.000	1.000	1.614	0.217	0.850	0.258	1.000	3.256	0.052	0.680	0.048	0.557
		LF	2.387	0.100	12.337	0.001	0.560	0.574	1.000	0.301	0.346	1.419	0.258	1.000	0.965	0.309	1.583	0.221	1.000	0.263	0.942
		LH	1.196	0.309	32.176	0.000	2.238	0.115	1.000	0.867	0.821	0.073	0.929	1.000	1.000	1.000	4.114	0.026	1.000	0.062	0.058
		FP	2.439	0.096	16.658	0.000	0.972	0.384	1.000	0.775	0.174	2.738	0.081	0.223	1.000	0.130	1.449	0.250	1.000	0.359	0.660
		HP	0.766	0.469	47.057	0.000	3.530	0.035	1.000	1.000	1.000	0.512	0.605	1.000	0.960	1.000	4.028	0.028	1.000	0.035	0.144

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A39: ANOVA table (1) of CW testing of young Pink1 x DAT mice: temporal parameters

				Pink1 x DAT, young, CW									males					females				
				Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
				F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
temporal parameters	1	Cadence [steps/sec]		2.813	0.068	6.866	0.011	0.370	0.692	0.047	0.607	0.659	1.766	0.187	0.256	0.467	1.000	1.368	0.270	0.369	1.000	0.652
	2	Stance duration [sec]	RF	2.518	0.089	10.450	0.002	0.032	0.968	0.055	0.625	0.716	2.198	0.128	0.134	0.696	1.000	0.776	0.469	0.667	1.000	1.000
			RH	4.282	0.018	9.033	0.004	0.716	0.492	0.028	0.038	1.000	6.828	0.003	0.018	0.005	1.000	0.678	0.515	0.806	1.000	1.000
			LF	1.913	0.156	11.678	0.001	0.014	0.986	0.109	0.708	0.995	1.470	0.245	0.291	0.966	1.000	0.643	0.533	0.806	1.000	1.000
			LH	2.179	0.122	4.298	0.042	0.569	0.569	0.116	0.443	1.000	1.952	0.158	0.319	0.254	1.000	0.800	0.459	0.692	1.000	1.000
			FP	2.390	0.100	11.863	0.001	0.026	0.974	0.062	0.614	0.788	1.941	0.160	0.175	0.765	1.000	0.769	0.472	0.674	1.000	1.000
			HP	3.711	0.030	7.978	0.006	0.741	0.481	0.033	0.090	1.000	4.410	0.020	0.063	0.031	1.000	0.810	0.454	0.638	1.000	1.000
	3	Stand [sec]	RF	2.741	0.072	1.941	0.168	0.213	0.809	0.065	1.000	0.331	1.317	0.282	0.344	1.000	1.000	1.622	0.214	0.336	1.000	0.413
			RH	0.673	0.514	0.027	0.871	0.079	0.924	0.803	1.000	1.000	0.469	0.630	1.000	1.000	1.000	0.244	0.785	1.000	1.000	1.000
			LF	2.805	0.068	2.427	0.124	0.188	0.829	0.057	1.000	0.362	1.444	0.251	0.298	1.000	1.000	1.507	0.237	0.349	1.000	0.502
			LH	0.800	0.454	0.510	0.478	0.074	0.929	0.725	1.000	1.000	0.386	0.683	1.000	1.000	1.000	0.493	0.616	1.000	1.000	1.000
			FP	2.835	0.066	2.240	0.139	0.200	0.819	0.057	1.000	0.335	1.396	0.262	0.315	1.000	1.000	1.612	0.216	0.325	1.000	0.441
			HP	0.751	0.476	0.184	0.670	0.085	0.918	0.711	1.000	1.000	0.443	0.646	1.000	1.000	1.000	0.371	0.693	1.000	1.000	1.000
	4	Stand index [cm/sec]	RF	2.466	0.093	6.660	0.012	0.603	0.550	0.074	0.477	1.000	1.459	0.247	0.433	0.443	1.000	1.588	0.221	0.302	1.000	0.521
			RH	0.395	0.675	2.244	0.139	0.247	0.782	1.000	1.000	1.000	0.348	0.709	1.000	1.000	1.000	0.320	0.728	1.000	1.000	1.000
			LF	2.453	0.094	9.548	0.003	0.971	0.384	0.064	0.607	0.811	2.634	0.087	0.117	0.244	1.000	0.639	0.534	1.000	1.000	0.933
			LH	0.386	0.681	7.845	0.007	0.557	0.576	0.936	1.000	1.000	0.651	0.528	0.787	1.000	1.000	0.099	0.906	1.000	1.000	1.000
			FP	2.999	0.057	9.527	0.003	0.883	0.418	0.038	0.413	0.811	2.227	0.124	0.195	0.263	1.000	1.390	0.264	0.415	1.000	0.513
			HP	0.236	0.790	6.705	0.012	0.609	0.547	1.000	1.000	1.000	0.613	0.548	0.880	1.000	1.000	0.072	0.930	1.000	1.000	1.000
	5	Body speed [cm/sec]	RF	2.989	0.058	4.003	0.050	0.538	0.586	0.113	0.092	1.000	1.455	0.248	1.000	0.295	1.000	2.138	0.135	0.156	0.560	1.000
			RH	3.065	0.054	3.892	0.053	0.453	0.638	0.125	0.076	1.000	1.832	0.177	0.787	0.197	1.000	1.614	0.215	0.280	0.663	1.000
			LF	3.112	0.051	3.441	0.068	0.507	0.605	0.101	0.086	1.000	1.556	0.227	0.903	0.265	1.000	2.068	0.144	0.169	0.570	1.000
			LH	3.515	0.036	4.076	0.048	0.554	0.577	0.068	0.064	1.000	1.926	0.162	0.677	0.182	1.000	2.093	0.140	0.160	0.604	1.000
			FP	3.059	0.054	3.724	0.058	0.524	0.595	0.106	0.088	1.000	1.505	0.237	0.953	0.280	1.000	2.113	0.138	0.160	0.563	1.000
			HP	3.288	0.044	3.987	0.050	0.503	0.607	0.092	0.070	1.000	1.881	0.169	0.729	0.188	1.000	1.849	0.174	0.212	0.632	1.000
	6	Body speed variation [%]	RF	2.769	0.070	0.001	0.973	0.518	0.598	0.826	0.068	0.704	2.589	0.091	1.000	0.095	0.512	0.574	0.569	1.000	0.921	1.000
			RH	1.138	0.327	0.177	0.676	0.374	0.689	1.000	0.651	0.570	1.143	0.332	1.000	0.525	0.713	0.226	0.799	1.000	1.000	1.000
			LF	2.737	0.072	0.224	0.638	1.247	0.294	1.000	0.094	0.257	3.507	0.042	1.000	0.059	0.140	0.170	0.844	1.000	1.000	1.000
			LH	3.318	0.043	0.665	0.418	0.606	0.549	0.618	0.038	0.648	3.194	0.054	0.772	0.051	0.515	0.596	0.557	1.000	0.859	1.000
			FP	2.910	0.062	0.050	0.824	0.906	0.409	1.000	0.064	0.395	3.079	0.060	1.000	0.069	0.263	0.388	0.681	1.000	1.000	1.000
			HP	2.004	0.143	0.364	0.548	0.514	0.600	1.000	0.170	0.572	2.027	0.148	1.000	0.172	0.584	0.283	0.756	1.000	1.000	1.000
	7	Number of steps [#]		0.396	0.675	0.793	0.377	0.297	0.744	1.000	1.000	1.000	0.145	0.865	1.000	1.000	1.000	0.539	0.589	0.937	1.000	1.000
	8	Swing duration [sec]	RF	0.997	0.375	19.342	0.000	0.119	0.888	0.721	0.447	1.000	0.659	0.524	0.966	0.980	1.000	0.477	0.625	1.000	1.000	1.000
			RH	1.984	0.146	28.826	0.000	0.169	0.845	0.080	0.889	0.645	1.152	0.329	0.442	0.914	1.000	0.961	0.394	0.577	1.000	0.911
			LF	1.220	0.302	19.422	0.000	0.034	0.967	0.431	0.397	1.000	0.932	0.404	0.726	0.729	1.000	0.428	0.656	1.000	1.000	1.000
			LH	3.303	0.043	54.423	0.000	0.927	0.401	0.022	0.111	1.000	4.415	0.020	0.048	0.038	1.000	0.504	0.609	0.971	1.000	1.000
			FP	1.242	0.296	21.747	0.000	0.077	0.926	0.479	0.353	1.000	0.877	0.426	0.768	0.770	1.000	0.492	0.616	1.000	0.990	1.000
			HP	2.859	0.065	48.128	0.000	0.483	0.619	0.026	0.294	0.860	2.473	0.100	0.146	0.240	1.000	0.847	0.438	0.627	1.000	1.000
	9	Swing speed [cm/sec]	RF	2.499	0.090	11.171	0.001	0.397	0.674	0.525	0.073	1.000	1.087	0.349	1.000	0.534	0.797	2.024	0.149	0.371	0.223	1.000
			RH	2.557	0.086	23.462	0.000	0.526	0.593	0.080	0.169	1.000	0.981	0.386	1.000	0.526	1.000	2.362	0.111	0.115	0.682	0.993
			LF	2.469	0.093	8.374	0.005	0.552	0.578	0.348	0.084	1.000	0.839	0.441	1.000	0.682	1.000	2.398	0.108	0.218	0.198	1.000
			LH	6.095	0.004	35.509	0.000	0.661	0.520	0.007	0.006	1.000	3.763	0.034	0.218	0.034	1.000	2.779	0.078	0.096	0.296	1.000
			FP	2.605	0.082	10.192	0.002	0.490	0.615	0.393	0.068	1.000	1.006	0.377	1.000	0.571	0.886	2.358	0.111	0.250	0.186	1.000
			HP	4.541	0.014	33.011	0.000	0.571	0.568	0.017	0.024	1.000	2.262	0.121	0.499	0.133	1.000	2.905	0.070	0.076	0.363	1.000
	10	Run duration [sec]		2.164	0.123	1.840	0.180	0.411	0.665	0.154	0.314	1.000	0.997	0.380	0.972	0.539	1.000	1.405	0.260	0.319	1.000	1.000
	11	Run speed [cm/sec]		2.778	0.070	3.762	0.057	0.427	0.654	0.138	0.109	1.000	1.638	0.210	0.789	0.247	1.000	1.498	0.239	0.304	0.788	1.000
	12	Run maximum variation [%]		1.777	0.177	1.293	0.260	0.428	0.653	1.000	0.194	0.883	3.153	0.056	1.000	0.058	0.340	0.182	0.835	1.000	1.000	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A40: ANOVA table (2) of CW testing of young Pink1 x DAT mice: comparative paws

			Pink1 x DAT, young, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
comparative paws	13 Stride length [cm]	RF	1.143	0.325	0.084	0.772	1.319	0.275	0.996	0.400	1.000	0.471	0.629	1.000	1.000	1.000	2.238	0.124	0.180	0.330	1.000
		RH	1.255	0.292	0.059	0.809	1.372	0.261	0.820	0.369	1.000	0.467	0.631	1.000	1.000	1.000	2.369	0.110	0.149	0.338	1.000
		LF	1.108	0.337	0.023	0.881	1.609	0.208	1.000	0.399	1.000	0.531	0.593	1.000	1.000	0.948	2.303	0.117	0.197	0.259	1.000
		LH	1.431	0.247	0.008	0.930	1.506	0.230	0.625	0.328	1.000	0.216	0.807	1.000	1.000	1.000	2.863	0.072	0.117	0.184	1.000
		FP	1.135	0.328	0.049	0.825	1.471	0.238	1.000	0.394	1.000	0.493	0.616	1.000	1.000	0.986	2.299	0.117	0.183	0.284	1.000
		HP	1.353	0.266	0.005	0.943	1.420	0.249	0.709	0.343	1.000	0.331	0.720	1.000	1.000	1.000	2.618	0.089	0.131	0.248	1.000
	14 Step cycle [sec]	RF	2.165	0.123	7.773	0.007	0.033	0.968	0.088	0.923	0.664	1.217	0.309	0.387	1.000	1.000	0.977	0.388	0.525	1.000	1.000
		RH	2.143	0.126	7.648	0.007	0.100	0.905	0.092	0.706	0.896	1.413	0.258	0.328	0.777	1.000	0.837	0.443	0.623	1.000	1.000
		LF	2.147	0.125	7.919	0.007	0.045	0.956	0.090	0.822	0.760	1.243	0.302	0.381	1.000	1.000	0.948	0.399	0.541	1.000	1.000
		LH	2.289	0.110	8.961	0.004	0.293	0.747	0.078	0.633	0.890	1.811	0.180	0.243	0.462	1.000	0.828	0.446	0.664	1.000	1.000
		FP	2.167	0.123	7.860	0.007	0.039	0.961	0.088	0.869	0.707	1.232	0.305	0.382	1.000	1.000	0.970	0.390	0.528	1.000	1.000
		HP	2.234	0.116	8.344	0.005	0.187	0.830	0.083	0.662	0.888	1.615	0.215	0.279	0.597	1.000	0.833	0.444	0.639	1.000	1.000
	15 Duty cycle [%]	RF	3.601	0.033	1.326	0.254	0.385	0.682	0.091	1.000	0.076	0.898	0.417	0.641	1.000	0.979	3.432	0.045	0.137	1.000	0.058
		RH	0.090	0.914	5.669	0.020	0.027	0.974	1.000	1.000	1.000	0.076	0.927	1.000	1.000	1.000	0.034	0.966	1.000	1.000	1.000
		LF	2.245	0.114	0.258	0.613	0.231	0.795	0.199	1.000	0.271	0.714	0.497	0.743	1.000	1.000	1.858	0.173	0.378	1.000	0.249
		LH	0.217	0.806	17.825	0.000	0.288	0.751	1.000	1.000	1.000	0.300	0.743	1.000	1.000	1.000	0.170	0.844	1.000	1.000	1.000
		FP	3.348	0.042	0.799	0.375	0.335	0.717	0.094	1.000	0.103	0.862	0.432	0.645	1.000	1.000	3.312	0.050	0.137	1.000	0.067
		HP	0.007	0.993	11.850	0.001	0.088	0.916	1.000	1.000	1.000	0.021	0.980	1.000	1.000	1.000	0.088	0.916	1.000	1.000	1.000
	16 Base of support [cm]	FP	1.409	0.252	2.523	0.117	0.115	0.891	0.396	1.000	0.700	0.635	0.537	1.000	1.000	0.947	0.924	0.408	0.552	1.000	1.000
		HP	1.162	0.320	5.555	0.022	4.579	0.014	0.412	0.564	1.000	4.504	0.019	0.016	0.276	0.623	1.081	0.352	0.677	1.000	0.568
	17 Initial dual stance [sec]	RF	1.171	0.317	0.557	0.458	0.283	0.754	0.768	1.000	0.513	0.296	0.746	1.000	1.000	1.000	1.635	0.211	0.900	1.000	0.242
		RH	0.903	0.411	3.660	0.060	0.391	0.678	1.000	0.695	0.813	0.717	0.496	1.000	1.000	0.793	0.423	0.659	1.000	1.000	1.000
		LF	3.358	0.041	0.727	0.397	0.523	0.595	0.076	1.000	0.138	0.608	0.550	0.864	1.000	1.000	3.996	0.029	0.056	1.000	0.055
		LH	0.261	0.771	2.832	0.097	0.597	0.553	1.000	1.000	1.000	0.466	0.632	1.000	1.000	1.000	0.420	0.661	1.000	1.000	1.000
		FP	2.376	0.101	0.739	0.393	0.387	0.680	0.220	1.000	0.215	0.517	0.601	0.975	1.000	1.000	2.961	0.067	0.211	1.000	0.081
		HP	0.199	0.820	4.032	0.049	0.099	0.906	1.000	1.000	1.000	0.177	0.838	1.000	1.000	1.000	0.026	0.975	1.000	1.000	1.000
	18 Terminal dual stance [sec]	RF	3.063	0.054	0.499	0.483	0.650	0.526	0.085	1.000	0.209	0.463	0.633	1.000	1.000	1.000	3.834	0.033	0.053	1.000	0.076
		RH	0.383	0.683	3.838	0.055	0.625	0.538	1.000	1.000	0.987	0.520	0.599	1.000	1.000	1.000	0.567	0.573	1.000	0.978	1.000
		LF	0.994	0.376	0.530	0.469	0.469	0.628	0.924	1.000	0.620	0.231	0.795	1.000	1.000	1.000	1.905	0.166	0.988	0.967	0.181
		LH	0.717	0.492	3.488	0.066	0.165	0.848	1.000	0.844	0.996	0.400	0.673	1.000	1.000	1.000	0.767	0.473	1.000	0.674	1.000
		FP	1.912	0.156	0.620	0.434	0.518	0.598	0.324	1.000	0.327	0.339	0.715	1.000	1.000	1.000	2.948	0.067	0.230	1.000	0.078
		HP	0.140	0.869	4.194	0.045	0.029	0.971	1.000	1.000	1.000	0.087	0.917	1.000	1.000	1.000	0.074	0.929	1.000	1.000	1.000
	19 Print position right paws [cm]		0.744	0.479	4.200	0.045	0.735	0.483	1.000	1.000	0.901	0.050	0.951	1.000	1.000	1.000	1.597	0.219	1.000	0.983	0.255
	20 Print position left paws [cm]		0.370	0.692	1.706	0.196	0.878	0.421	1.000	1.000	1.000	0.560	0.576	0.917	1.000	1.000	0.733	0.489	1.000	1.000	0.710

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A41: ANOVA table (3) of CW testing of young Pink1 x DAT mice: interlimb coordination and individual paw

			Pink1 x DAT, young, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
interlimb coordination	21	Number of patterns	0.322	0.726	0.570	0.453	0.326	0.723	1.000	1.000	1.000	0.302	0.741	1.000	1.000	1.000	0.336	0.717	1.000	1.000	1.000
	22	Regularity index [%]	0.916	0.405	0.718	0.400	0.643	0.529	0.722	1.000	0.743	1.117	0.340	1.000	1.000	0.435	0.489	0.618	1.000	1.000	1.000
	23	Phase dispersion [%] RF-LH	0.988	0.378	3.674	0.060	0.985	0.379	0.932	0.517	1.000	0.972	0.389	0.869	0.591	1.000	1.734	0.193	0.681	1.000	0.225
		LF-RH	0.951	0.392	0.118	0.732	0.505	0.606	1.000	0.561	1.000	0.457	0.637	1.000	1.000	1.000	1.159	0.327	1.000	0.844	0.470
		LH-RH	1.935	0.153	0.331	0.567	1.467	0.238	1.000	0.477	0.163	2.670	0.085	0.712	0.899	0.083	0.454	0.639	1.000	1.000	1.000
		LF-RF	0.041	0.960	0.025	0.874	0.025	0.975	1.000	1.000	1.000	0.038	0.963	1.000	1.000	1.000	0.029	0.972	1.000	1.000	1.000
		RF-RH	1.819	0.171	1.293	0.260	1.079	0.346	0.459	1.000	0.236	0.119	0.888	1.000	1.000	1.000	3.245	0.053	0.109	1.000	0.087
		LF-LH	6.530	0.003	2.795	0.100	0.034	0.967	0.029	1.000	0.002	2.976	0.065	0.360	1.000	0.069	3.868	0.032	0.122	1.000	0.037
	24	Couplings [%] RF-LH	1.358	0.265	1.836	0.180	0.658	0.522	1.000	0.404	0.675	0.908	0.413	1.000	0.562	1.000	1.955	0.159	0.891	1.000	0.171
		LF-RH	1.383	0.258	1.091	0.300	0.971	0.384	0.351	1.000	0.676	0.031	0.969	1.000	1.000	1.000	2.342	0.113	0.153	1.000	0.278
		LH-RF	3.157	0.049	0.831	0.366	0.107	0.899	0.462	0.790	0.037	1.621	0.214	0.792	1.000	0.257	1.787	0.184	1.000	0.800	0.213
		RH-LF	1.052	0.355	0.656	0.421	0.950	0.392	0.634	1.000	0.680	0.006	0.994	1.000	1.000	1.000	1.804	0.181	0.285	1.000	0.352
		LH-RH	0.857	0.430	0.017	0.898	1.049	0.356	1.000	1.000	0.507	1.517	0.235	0.553	1.000	0.348	0.312	0.734	1.000	1.000	1.000
		LF-RF	0.071	0.931	0.034	0.854	0.018	0.982	1.000	1.000	1.000	0.069	0.933	1.000	1.000	1.000	0.024	0.977	1.000	1.000	1.000
		RH-LH	1.180	0.314	0.049	0.826	0.664	0.518	1.000	0.976	0.356	1.501	0.238	0.739	1.000	0.306	0.423	0.659	1.000	1.000	1.000
		RF-LF	0.122	0.885	0.005	0.944	0.021	0.979	1.000	1.000	1.000	0.082	0.922	1.000	1.000	1.000	0.061	0.941	1.000	1.000	1.000
		RF-RH	1.725	0.187	1.209	0.276	1.076	0.347	0.541	1.000	0.250	0.107	0.899	1.000	1.000	1.000	3.170	0.056	0.130	1.000	0.084
		LF-LH	6.951	0.002	1.338	0.252	0.060	0.942	0.018	1.000	0.002	3.493	0.042	0.174	1.000	0.053	3.693	0.036	0.170	1.000	0.039
		RH-RF	1.469	0.238	1.115	0.295	0.838	0.437	0.644	1.000	0.326	0.051	0.950	1.000	1.000	1.000	2.592	0.091	0.252	1.000	0.116
		LH-LF	5.873	0.005	1.550	0.218	0.347	0.708	0.019	1.000	0.006	2.604	0.090	0.137	1.000	0.226	4.045	0.027	0.219	0.961	0.025
individual paw	25	% Max contact at [%] RF	0.978	0.382	0.564	0.455	0.455	0.636	1.000	1.000	0.566	0.197	0.822	1.000	1.000	1.000	1.174	0.322	1.000	1.000	0.417
		RH	1.453	0.242	0.541	0.465	0.097	0.908	1.000	1.000	0.298	0.455	0.639	1.000	1.000	1.000	1.257	0.299	1.000	1.000	0.382
		LF	0.255	0.775	4.168	0.045	0.011	0.989	1.000	1.000	1.000	0.171	0.844	1.000	1.000	1.000	0.089	0.915	1.000	1.000	1.000
		LH	2.226	0.116	0.482	0.490	2.155	0.124	1.000	0.457	0.099	2.074	0.142	0.340	1.000	0.215	2.931	0.068	0.566	0.066	1.000
		FP	0.755	0.474	2.995	0.088	0.165	0.848	1.000	1.000	0.899	0.258	0.774	1.000	1.000	1.000	0.693	0.508	1.000	1.000	0.749
		HP	2.428	0.096	0.000	0.990	0.913	0.407	1.000	0.572	0.085	1.448	0.250	0.584	1.000	0.372	2.550	0.094	1.000	0.120	0.328
		RF	0.084	0.920	38.611	0.000	0.600	0.552	1.000	1.000	1.000	0.415	0.664	1.000	1.000	1.000	0.203	0.818	1.000	1.000	1.000
		RH	0.331	0.720	18.373	0.000	1.921	0.155	1.000	1.000	1.000	1.074	0.354	0.588	0.682	1.000	1.215	0.310	1.000	0.408	0.991
		LF	0.125	0.883	26.898	0.000	0.536	0.588	1.000	1.000	1.000	0.119	0.888	1.000	1.000	1.000	0.731	0.490	1.000	0.708	1.000
		LH	0.809	0.450	27.938	0.000	0.343	0.711	1.000	1.000	0.838	0.482	0.622	1.000	1.000	1.000	0.833	0.444	1.000	0.839	0.817
		FP	0.036	0.964	34.903	0.000	0.598	0.553	1.000	1.000	1.000	0.254	0.777	1.000	1.000	1.000	0.466	0.632	1.000	1.000	1.000
		HP	0.601	0.551	25.692	0.000	1.074	0.348	1.000	1.000	1.000	0.709	0.500	0.735	1.000	1.000	1.110	0.342	1.000	0.518	0.809
	26	Max contact max intensity [pixel] RF	0.195	0.823	26.549	0.000	0.551	0.579	1.000	1.000	1.000	0.113	0.893	1.000	1.000	1.000	0.983	0.386	1.000	1.000	0.547
		RH	0.352	0.704	25.652	0.000	1.136	0.328	1.000	1.000	1.000	0.838	0.442	0.635	1.000	1.000	0.656	0.526	1.000	0.799	1.000
		LF	0.291	0.749	21.098	0.000	0.812	0.449	1.000	1.000	1.000	0.409	0.668	1.000	1.000	1.000	0.874	0.427	1.000	0.844	0.750
		LH	0.554	0.577	33.353	0.000	0.183	0.833	1.000	0.858	1.000	0.606	0.551	1.000	1.000	0.922	0.151	0.860	1.000	1.000	1.000
		FP	0.262	0.770	26.355	0.000	0.717	0.492	1.000	1.000	1.000	0.257	0.775	1.000	1.000	1.000	1.079	0.352	1.000	0.846	0.530
		HP	0.410	0.666	33.148	0.000	0.413	0.663	1.000	1.000	1.000	0.406	0.670	1.000	1.000	1.000	0.455	0.638	1.000	1.000	1.000
	27	Max contact mean intensity [pixel] RF	0.175	0.840	15.302	0.000	0.493	0.613	1.000	1.000	1.000	0.437	0.650	1.000	1.000	1.000	0.077	0.926	1.000	1.000	1.000
		RH	0.804	0.452	14.698	0.000	0.065	0.937	1.000	1.000	0.774	0.630	0.539	1.000	1.000	0.834	0.297	0.745	1.000	1.000	1.000
		LF	0.300	0.742	16.304	0.000	0.644	0.529	1.000	1.000	1.000	0.053	0.948	1.000	1.000	1.000	1.972	0.156	1.000	0.339	0.258
		LH	0.622	0.540	15.407	0.000	0.236	0.791	0.789	0.888	1.000	0.565	0.574	1.000	0.887	1.000	0.295	0.747	1.000	1.000	1.000
		FP	0.107	0.898	18.041	0.000	0.559	0.575	1.000	1.000	1.000	0.207	0.814	1.000	1.000	1.000	0.736	0.487	1.000	0.875	0.961
		HP	0.577	0.564	18.750	0.000	0.098	0.906	1.000	0.879	1.000	0.516	0.602	1.000	1.000	1.000	0.177	0.839	1.000	1.000	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A42: ANOVA table (4) of CW testing of young Pink1 x DAT mice: individual paw

			Pink1 x DAT, young, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
28	Maximum intensity [pixel]	RF	0.219	0.804	27.636	0.000	0.509	0.604	1.000	1.000	1.000	0.103	0.903	1.000	1.000	1.000	1.070	0.355	1.000	1.000	0.462
		RH	0.510	0.603	24.408	0.000	1.433	0.246	1.000	1.000	1.000	1.346	0.275	0.354	0.813	1.000	0.629	0.540	1.000	0.844	1.000
		LF	0.163	0.850	25.118	0.000	0.723	0.489	1.000	1.000	1.000	0.282	0.756	1.000	1.000	1.000	0.823	0.448	1.000	1.000	0.675
		LH	0.620	0.541	32.344	0.000	0.128	0.880	1.000	0.898	1.000	0.512	0.604	1.000	1.000	0.963	0.281	0.757	1.000	1.000	1.000
		FP	0.206	0.814	29.014	0.000	0.662	0.519	1.000	1.000	1.000	0.199	0.821	1.000	1.000	1.000	1.091	0.349	1.000	1.000	0.454
		HP	0.516	0.600	31.740	0.000	0.648	0.526	1.000	1.000	1.000	0.641	0.533	0.839	1.000	1.000	0.558	0.578	1.000	0.922	1.000
29	% Maximum intensity at [sec]	RF	0.615	0.544	25.504	0.000	0.794	0.456	1.000	1.000	0.690	0.477	0.625	1.000	1.000	1.000	1.057	0.360	1.000	0.477	1.000
		RH	0.274	0.761	4.073	0.048	2.078	0.134	1.000	1.000	1.000	0.609	0.550	1.000	0.836	1.000	2.139	0.135	1.000	0.261	0.251
		LF	0.132	0.877	7.390	0.008	2.412	0.098	1.000	1.000	1.000	1.875	0.170	0.186	1.000	0.919	0.716	0.497	0.739	1.000	1.000
		LH	0.920	0.404	8.394	0.005	1.634	0.203	0.872	0.566	1.000	2.127	0.136	0.685	0.145	1.000	0.106	0.900	1.000	1.000	1.000
		FP	0.270	0.764	18.631	0.000	1.931	0.153	1.000	1.000	0.995	1.200	0.314	0.416	1.000	0.964	1.049	0.362	0.689	0.648	1.000
		HP	0.394	0.676	7.379	0.009	2.171	0.122	1.000	1.000	1.000	1.482	0.242	0.973	0.287	1.000	0.904	0.415	1.000	0.799	0.743
30	Minimum intensity [pixel]	RF	0.669	0.516	12.638	0.001	1.507	0.229	1.000	1.000	1.000	0.121	0.887	1.000	1.000	1.000	1.607	0.217	0.270	1.000	0.631
		RH	0.423	0.657	18.037	0.000	2.307	0.108	1.000	1.000	1.000	2.731	0.080	0.317	0.091	1.000	0.359	0.701	1.000	1.000	1.000
		LF	1.298	0.280	2.655	0.108	0.908	0.408	0.616	0.539	1.000	2.142	0.134	0.263	0.223	1.000	0.020	0.981	1.000	1.000	1.000
		LH	0.402	0.671	26.393	0.000	0.122	0.885	1.000	1.000	1.000	1.031	0.368	0.482	1.000	1.000	0.047	0.954	1.000	1.000	1.000
		FP	0.995	0.375	10.331	0.002	0.413	0.663	0.722	1.000	1.000	0.389	0.681	1.000	1.000	1.000	0.858	0.434	0.618	1.000	1.000
		HP	0.419	0.659	28.958	0.000	1.595	0.211	1.000	1.000	1.000	2.653	0.086	0.208	0.127	1.000	0.149	0.862	1.000	1.000	1.000
31	Mean intensity mean [pixel]	RF	0.250	0.780	11.070	0.001	0.744	0.479	1.000	1.000	1.000	0.578	0.567	0.948	1.000	1.000	0.332	0.720	1.000	1.000	1.000
		RH	1.195	0.310	12.840	0.001	0.073	0.930	1.000	1.000	0.473	0.938	0.402	1.000	1.000	0.566	0.448	0.643	1.000	1.000	1.000
		LF	0.279	0.757	16.780	0.000	0.616	0.543	1.000	1.000	1.000	0.169	0.845	1.000	1.000	1.000	1.187	0.319	1.000	0.888	0.441
		LH	0.575	0.565	16.711	0.000	0.146	0.865	1.000	0.757	1.000	0.337	0.716	1.000	1.000	1.000	0.391	0.680	1.000	1.000	1.000
		FP	0.285	0.753	15.682	0.000	0.630	0.536	1.000	1.000	1.000	0.346	0.710	1.000	1.000	1.000	0.757	0.478	1.000	0.951	0.848
		HP	0.936	0.398	17.797	0.000	0.100	0.905	1.000	0.752	0.789	0.678	0.515	1.000	1.000	0.764	0.404	0.671	1.000	1.000	1.000
32	Intensity of 15 most intense pixel [pixel]	RF	0.246	0.783	21.132	0.000	0.659	0.521	1.000	1.000	1.000	0.299	0.744	1.000	1.000	1.000	0.774	0.470	1.000	0.941	0.827
		RH	0.553	0.578	19.702	0.000	1.121	0.332	1.000	1.000	1.000	0.853	0.435	0.627	1.000	1.000	0.830	0.446	1.000	0.725	0.983
		LF	0.201	0.819	19.772	0.000	1.011	0.370	1.000	1.000	1.000	0.290	0.750	1.000	1.000	1.000	1.326	0.280	1.000	0.529	0.501
		LH	0.757	0.473	29.724	0.000	0.139	0.870	1.000	0.837	1.000	0.331	0.721	1.000	1.000	1.000	0.614	0.548	1.000	0.889	1.000
		FP	0.234	0.792	21.942	0.000	0.865	0.426	1.000	1.000	1.000	0.299	0.744	1.000	1.000	1.000	1.135	0.334	1.000	0.653	0.581
		HP	0.638	0.532	26.262	0.000	0.580	0.563	1.000	1.000	1.000	0.432	0.653	1.000	1.000	1.000	0.849	0.437	1.000	0.694	0.999
33	Print length [cm]	RF	0.202	0.818	9.381	0.003	0.634	0.534	1.000	1.000	1.000	0.101	0.905	1.000	1.000	1.000	0.673	0.518	1.000	0.794	1.000
		RH	0.304	0.739	19.008	0.000	1.214	0.304	1.000	1.000	1.000	0.620	0.544	0.877	1.000	1.000	0.925	0.407	1.000	0.570	1.000
		LF	0.983	0.380	2.950	0.091	0.345	0.709	1.000	0.464	1.000	0.089	0.915	1.000	1.000	1.000	1.531	0.232	1.000	0.270	1.000
		LH	1.181	0.314	29.451	0.000	0.497	0.611	1.000	0.573	0.738	0.127	0.881	1.000	1.000	1.000	1.154	0.329	1.000	0.759	0.503
		FP	0.552	0.579	6.279	0.015	0.501	0.608	1.000	0.842	1.000	0.018	0.983	1.000	1.000	1.000	1.116	0.340	1.000	0.442	1.000
		HP	0.713	0.494	28.284	0.000	0.794	0.457	1.000	1.000	1.000	0.225	0.800	1.000	1.000	1.000	1.126	0.337	1.000	0.560	0.696
34	Print Width [cm]	RF	0.389	0.679	20.112	0.000	0.125	0.882	1.000	1.000	1.000	0.072	0.930	1.000	1.000	1.000	0.447	0.644	1.000	1.000	1.000
		RH	0.765	0.470	17.674	0.000	2.117	0.129	0.995	1.000	1.000	1.901	0.166	0.280	0.313	1.000	0.821	0.449	1.000	0.764	0.931
		LF	0.599	0.553	8.715	0.004	1.393	0.256	1.000	1.000	0.949	1.422	0.256	0.306	1.000	1.000	0.682	0.513	1.000	0.758	1.000
		LH	2.310	0.108	26.161	0.000	0.431	0.652	0.353	1.000	0.269	1.678	0.203	0.258	1.000	0.636	1.006	0.377	1.000	1.000	0.498
		FP	0.414	0.663	17.127	0.000	0.481	0.620	1.000	1.000	1.000	0.262	0.771	1.000	1.000	1.000	0.631	0.539	1.000	0.837	1.000
		HP	1.593	0.211	24.516	0.000	1.221	0.302	0.510	1.000	0.644	1.844	0.175	0.196	0.737	1.000	0.878	0.426	1.000	1.000	0.635
35	Print area [cm]	RF	0.154	0.858	23.206	0.000	0.949	0.393	1.000	1.000	1.000	0.279	0.759	1.000	1.000	1.000	0.932	0.405	1.000	0.679	0.839
		RH	0.334	0.717	17.657	0.000	1.955	0.150	1.000	1.000	1.000	1.188	0.318	0.505	0.642	1.000	1.092	0.348	1.000	0.460	1.000
		LF	0.325	0.724	16.016	0.000	1.252	0.293	1.000	1.000	1.000	0.249	0.781	1.000	1.000	1.000	1.656	0.207	1.000	0.255	0.732
		LH	0.929	0.400	28.645	0.000	0.394	0.676	1.000	1.000	0.732	0.488	0.618	1.000	1.000	1.000	0.994	0.381	1.000	0.748	0.665
		FP	0.228	0.797	21.027	0.000	1.153	0.322	1.000	1.000	1.000	0.269	0.766	1.000	1.000	1.000	1.357	0.272	1.000	0.382	0.722
		HP	0.629	0.537	25.423	0.000	1.178	0.314	1.000	1.000	1.000	0.794	0.461	0.662	1.000	1.000	1.112	0.342	1.000	0.518	0.806

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A43: ANOVA table (1) of CW testing of mid-aged Pink1 x DAT mice: temporal parameters

				Pink1 x DAT, mid-aged, CW									males					females				
				Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
				F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
temporal parameters	1	Cadence [steps/sec]		0.043	0.958	4.033	0.049	0.184	0.833	1.000	1.000	1.000	0.049	0.953	1.000	1.000	1.000	0.165	0.849	1.000	1.000	1.000
	2	Stance duration [sec]	RF	0.171	0.843	10.963	0.002	2.115	0.130	1.000	1.000	1.000	0.838	0.443	0.715	1.000	0.991	1.324	0.281	0.350	1.000	0.931
			RH	3.390	0.041	18.203	0.000	0.385	0.682	0.041	1.000	0.061	5.181	0.012	0.017	1.000	0.063	0.563	0.576	1.000	1.000	1.000
			LF	0.101	0.904	8.566	0.005	0.538	0.587	1.000	1.000	1.000	0.153	0.859	1.000	1.000	1.000	0.533	0.592	1.000	1.000	0.982
			LH	0.682	0.509	11.734	0.001	3.073	0.054	1.000	1.000	0.517	3.186	0.057	0.137	1.000	0.100	0.850	0.437	0.629	1.000	1.000
			FP	0.070	0.933	10.683	0.002	1.271	0.288	1.000	1.000	1.000	0.409	0.668	1.000	1.000	1.000	0.898	0.418	0.641	1.000	0.921
			HP	1.918	0.156	18.288	0.000	1.802	0.174	0.273	1.000	0.121	5.252	0.012	0.023	1.000	0.036	0.127	0.882	1.000	1.000	1.000
	3	Stand [sec]	RF	0.181	0.835	1.855	0.178	0.061	0.941	1.000	1.000	1.000	0.095	0.910	1.000	1.000	1.000	0.140	0.870	1.000	1.000	1.000
			RH	0.751	0.477	0.468	0.496	0.178	0.837	0.812	1.000	1.000	0.617	0.547	0.828	1.000	1.000	0.326	0.724	1.000	1.000	1.000
			LF	0.036	0.965	1.302	0.259	0.073	0.930	1.000	1.000	1.000	0.090	0.915	1.000	1.000	1.000	0.010	0.990	1.000	1.000	1.000
			LH	1.260	0.291	0.066	0.798	0.216	0.806	0.415	1.000	0.761	0.409	0.668	1.000	1.000	1.000	1.092	0.349	0.641	1.000	0.594
			FP	0.096	0.909	1.600	0.211	0.051	0.951	1.000	1.000	1.000	0.096	0.908	1.000	1.000	1.000	0.046	0.955	1.000	1.000	1.000
			HP	1.028	0.364	0.227	0.636	0.160	0.852	0.564	1.000	0.868	0.514	0.604	0.960	1.000	1.000	0.672	0.518	1.000	1.000	0.853
	4	Stand index [cm/sec]	RF	0.936	0.398	0.275	0.602	0.524	0.595	1.000	0.664	0.861	1.125	0.339	1.000	1.000	0.439	0.450	0.642	1.000	1.000	1.000
			RH	0.415	0.663	6.462	0.014	0.118	0.889	1.000	1.000	1.000	0.044	0.957	1.000	1.000	1.000	0.779	0.468	1.000	1.000	0.776
			LF	0.104	0.901	0.036	0.850	0.413	0.664	1.000	1.000	1.000	0.461	0.635	1.000	1.000	1.000	0.129	0.879	1.000	1.000	1.000
			LH	2.212	0.119	1.581	0.214	0.285	0.753	0.136	0.944	0.886	0.533	0.593	0.948	1.000	1.000	1.915	0.165	0.185	1.000	0.671
			FP	0.492	0.614	0.150	0.700	0.549	0.581	1.000	1.000	1.000	0.834	0.445	1.000	1.000	0.627	0.315	0.732	1.000	1.000	1.000
			HP	1.057	0.354	4.334	0.042	0.244	0.784	0.645	1.000	0.901	0.140	0.870	1.000	1.000	1.000	1.452	0.250	0.432	1.000	0.444
	5	Body speed [cm/sec]	RF	1.326	0.273	0.953	0.333	0.519	0.598	1.000	0.881	0.438	1.607	0.218	1.000	0.428	0.359	0.225	0.800	1.000	1.000	1.000
			RH	1.096	0.341	0.665	0.418	0.522	0.596	1.000	1.000	0.539	1.411	0.261	1.000	0.482	0.450	0.246	0.783	1.000	1.000	1.000
			LF	1.107	0.337	0.815	0.371	0.691	0.505	1.000	1.000	0.540	1.570	0.226	1.000	0.422	0.387	0.230	0.796	1.000	1.000	1.000
			LH	1.329	0.273	0.557	0.459	0.785	0.461	1.000	0.967	0.422	1.913	0.166	1.000	0.309	0.292	0.258	0.774	1.000	1.000	1.000
			FP	1.206	0.307	0.887	0.350	0.607	0.548	1.000	1.000	0.491	1.587	0.222	1.000	0.426	0.373	0.218	0.805	1.000	1.000	1.000
			HP	1.214	0.305	0.608	0.439	0.649	0.526	1.000	1.000	0.475	1.660	0.208	1.000	0.386	0.361	0.252	0.779	1.000	1.000	1.000
	6	Body speed variation [%]	RF	0.521	0.597	0.001	0.970	0.922	0.403	1.000	1.000	1.000	0.125	0.883	1.000	1.000	1.000	1.214	0.311	0.392	1.000	1.000
			RH	1.359	0.265	0.265	0.609	1.449	0.243	1.000	1.000	0.340	1.600	0.220	1.000	0.270	0.729	1.164	0.326	0.460	1.000	0.786
			LF	0.221	0.803	0.003	0.956	1.551	0.221	1.000	1.000	1.000	1.049	0.364	1.000	0.497	1.000	0.802	0.458	0.868	0.824	1.000
			LH	1.135	0.328	0.380	0.540	2.332	0.106	0.965	1.000	0.471	2.429	0.107	1.000	0.165	0.241	1.128	0.337	0.484	0.843	1.000
			FP	0.367	0.694	0.000	0.995	1.153	0.323	1.000	1.000	1.000	0.473	0.628	1.000	1.000	1.000	0.956	0.396	0.566	1.000	1.000
			HP	1.315	0.276	0.351	0.556	1.894	0.160	1.000	1.000	0.363	2.131	0.138	1.000	0.182	0.383	1.123	0.339	0.433	1.000	1.000
	7	Number of steps [#]		2.959	0.060	8.137	0.006	2.892	0.064	0.224	1.000	0.053	3.110	0.060	1.000	0.230	0.072	2.259	0.122	0.140	0.482	1.000
	8	Swing duration [sec]	RF	0.681	0.510	11.190	0.001	0.454	0.637	1.000	1.000	1.000	0.255	0.777	1.000	1.000	1.000	1.039	0.366	1.000	1.000	0.483
			RH	1.477	0.237	16.878	0.000	2.860	0.065	0.325	1.000	0.230	3.858	0.033	0.041	1.000	0.157	0.331	0.721	1.000	1.000	1.000
			LF	0.448	0.641	16.944	0.000	1.904	0.158	1.000	1.000	1.000	0.482	0.622	1.000	1.000	1.000	1.596	0.220	0.330	1.000	0.460
			LH	5.050	0.010	25.847	0.000	0.778	0.464	0.005	1.000	0.045	5.455	0.010	0.008	0.444	0.262	1.133	0.336	0.623	1.000	0.564
			FP	0.505	0.606	15.355	0.000	1.116	0.335	1.000	1.000	1.000	0.349	0.709	1.000	1.000	1.000	1.278	0.293	0.662	1.000	0.424
			HP	3.514	0.036	25.745	0.000	2.008	0.144	0.029	1.000	0.059	5.379	0.011	0.010	0.843	0.137	0.336	0.717	1.000	1.000	1.000
	9	Swing speed [cm/sec]	RF	3.209	0.048	4.522	0.038	0.660	0.520	1.000	0.127	0.142	2.557	0.096	1.000	0.180	0.175	0.667	0.521	1.000	1.000	0.975
			RH	0.928	0.401	8.391	0.005	1.082	0.346	0.518	1.000	1.000	1.503	0.240	0.324	0.636	1.000	0.006	0.994	1.000	1.000	1.000
			LF	2.309	0.108	5.110	0.028	0.864	0.427	1.000	0.487	0.196	2.321	0.117	1.000	0.200	0.231	0.617	0.546	1.000	1.000	0.906
			LH	3.646	0.032	13.768	0.000	0.636	0.533	0.023	0.302	0.793	3.014	0.065	0.103	0.156	1.000	1.210	0.312	0.412	1.000	0.876
			FP	2.834	0.067	5.062	0.028	0.769	0.468	1.000	0.233	0.153	2.569	0.095	1.000	0.170	0.183	0.539	0.589	1.000	1.000	0.927
			HP	2.309	0.108	12.607	0.001	0.881	0.420	0.096	0.514	1.000	2.501	0.100	0.140	0.264	1.000	0.314	0.733	1.000	1.000	1.000
	10	Run duration [sec]		0.962	0.388	1.046	0.311	0.082	0.922	1.000	1.000	0.558	0.864	0.433	1.000	1.000	0.634	0.293	0.748	1.000	1.000	1.000
	11	Run speed [cm/sec]		0.663	0.519	1.465	0.231	0.306	0.738	1.000	1.000	0.957	0.704	0.503	1.000	0.860	1.000	0.213	0.809	1.000	1.000	1.000
	12	Run maximum variation [%]		0.866	0.426	1.751	0.191	0.528	0.592	1.000	0.536	1.000	0.025	0.975	1.000	1.000	1.000	1.225	0.308	0.864	0.417	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A44: ANOVA table (2) of CW testing of mid-aged Pink1 x DAT mice: comparative paws

			Pink1 x DAT, mid-aged, CW									males						females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value				Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2		F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
comparative paws	13	Stride length [cm]	RF	2.059	0.137	1.012	0.319	1.568	0.217	1.000	0.498	0.161	2.951	0.069	1.000	0.455	0.068		0.119	0.888	1.000	1.000	1.000
			RH	1.711	0.190	0.747	0.391	1.456	0.242	1.000	0.454	0.284	2.480	0.102	1.000	0.508	0.108		0.258	0.774	1.000	1.000	1.000
			LF	2.131	0.128	0.712	0.402	1.497	0.232	1.000	0.531	0.150	2.932	0.070	1.000	0.377	0.072		0.049	0.952	1.000	1.000	1.000
			LH	1.881	0.162	0.782	0.380	1.271	0.288	1.000	0.609	0.186	2.756	0.081	1.000	0.539	0.080		0.099	0.906	1.000	1.000	1.000
			FP	2.097	0.132	0.853	0.360	1.525	0.226	1.000	0.514	0.155	2.938	0.069	1.000	0.413	0.070		0.074	0.929	1.000	1.000	1.000
			HP	1.804	0.174	0.767	0.385	1.379	0.260	1.000	0.516	0.226	2.634	0.090	1.000	0.515	0.092		0.161	0.852	1.000	1.000	1.000
	14	Step cycle [sec]	RF	0.068	0.934	4.780	0.033	0.160	0.853	1.000	1.000	1.000	0.010	0.990	1.000	1.000	1.000		0.213	0.810	1.000	1.000	1.000
			RH	0.046	0.955	5.151	0.027	0.230	0.795	1.000	1.000	1.000	0.043	0.958	1.000	1.000	1.000		0.228	0.798	1.000	1.000	1.000
			LF	0.085	0.919	4.681	0.035	0.186	0.831	1.000	1.000	1.000	0.010	0.990	1.000	1.000	1.000		0.259	0.774	1.000	1.000	1.000
			LH	0.037	0.964	4.158	0.046	0.189	0.828	1.000	1.000	1.000	0.030	0.971	1.000	1.000	1.000		0.193	0.825	1.000	1.000	1.000
			FP	0.076	0.927	4.728	0.034	0.172	0.842	1.000	1.000	1.000	0.010	0.990	1.000	1.000	1.000		0.235	0.792	1.000	1.000	1.000
			HP	0.043	0.958	4.650	0.035	0.206	0.814	1.000	1.000	1.000	0.034	0.967	1.000	1.000	1.000		0.211	0.811	1.000	1.000	1.000
	15	Duty cycle [%]	RF	1.456	0.241	1.441	0.235	0.002	0.998	1.000	0.299	0.967	0.693	0.508	1.000	0.763	1.000		0.777	0.469	1.000	0.685	1.000
			RH	1.627	0.205	2.754	0.102	1.022	0.366	0.293	1.000	0.320	2.711	0.084	0.103	1.000	0.325		0.140	0.870	1.000	1.000	1.000
			LF	0.281	0.756	2.056	0.157	0.403	0.670	1.000	1.000	1.000	0.176	0.840	1.000	1.000	1.000		0.512	0.605	1.000	1.000	1.000
			LH	4.586	0.014	6.417	0.014	0.078	0.925	0.011	1.000	0.079	2.571	0.094	0.098	1.000	0.570		2.128	0.137	0.189	1.000	0.332
			FP	0.761	0.472	2.079	0.155	0.113	0.893	1.000	0.822	1.000	0.481	0.623	1.000	1.000	1.000		0.378	0.689	1.000	1.000	1.000
			HP	3.430	0.039	5.164	0.027	0.386	0.681	0.039	1.000	0.108	3.157	0.058	0.062	1.000	0.337		0.861	0.433	0.832	1.000	0.732
	16	Base of support [cm]	FP	0.713	0.494	0.103	0.749	0.486	0.618	0.812	1.000	1.000	0.815	0.453	1.000	1.000	0.639		0.448	0.643	1.000	1.000	1.000
			HP	3.457	0.038	8.092	0.006	6.748	0.002	0.032	0.285	1.000	8.405	0.001	0.001	0.035	0.641		0.343	0.712	1.000	1.000	1.000
	17	Initial dual stance [sec]	RF	0.175	0.840	0.067	0.796	0.698	0.502	1.000	1.000	1.000	0.643	0.533	0.911	1.000	1.000		0.213	0.809	1.000	1.000	1.000
			RH	2.827	0.067	1.047	0.310	0.309	0.735	0.088	1.000	0.157	2.020	0.151	0.190	1.000	0.511		0.922	0.409	0.837	1.000	0.653
			LF	0.539	0.586	0.268	0.607	0.473	0.626	1.000	1.000	1.000	0.841	0.442	1.000	0.703	0.963		0.175	0.840	1.000	1.000	1.000
			LH	2.024	0.141	1.549	0.218	0.368	0.693	0.251	1.000	0.197	1.213	0.313	0.423	1.000	0.925		1.179	0.322	1.000	1.000	0.406
			FP	0.170	0.844	0.173	0.679	0.339	0.714	1.000	1.000	1.000	0.317	0.731	1.000	1.000	1.000		0.183	0.833	1.000	1.000	1.000
			HP	2.790	0.070	1.425	0.237	0.377	0.688	0.106	1.000	0.126	1.850	0.176	0.222	1.000	0.584		1.153	0.329	0.906	1.000	0.441
	18	Terminal dual stance [sec]	RF	0.396	0.675	0.248	0.620	0.440	0.646	1.000	1.000	1.000	0.680	0.515	1.000	0.798	1.000		0.157	0.855	1.000	1.000	1.000
			RH	2.852	0.066	1.745	0.192	0.268	0.766	0.098	1.000	0.121	1.431	0.256	0.325	1.000	0.860		1.756	0.190	0.556	1.000	0.237
			LF	0.163	0.850	0.108	0.743	0.872	0.424	1.000	1.000	1.000	0.635	0.538	0.833	1.000	1.000		0.380	0.687	1.000	1.000	1.000
			LH	2.320	0.107	0.885	0.351	0.460	0.634	0.145	1.000	0.223	1.945	0.162	0.202	1.000	0.556		0.601	0.555	1.000	1.000	0.892
			FP	0.212	0.809	0.179	0.673	0.508	0.604	1.000	1.000	1.000	0.442	0.647	1.000	1.000	1.000		0.260	0.773	1.000	1.000	1.000
			HP	2.971	0.059	1.437	0.236	0.354	0.703	0.081	1.000	0.125	1.956	0.160	0.194	1.000	0.589		1.209	0.313	0.776	1.000	0.433
	19	Print position right paws [cm]		1.787	0.177	0.587	0.447	0.572	0.568	0.428	1.000	0.232	1.609	0.218	1.000	1.000	0.252		0.592	0.559	0.861	1.000	1.000
	20	Print position left paws [cm]		0.921	0.404	0.777	0.382	0.522	0.596	1.000	1.000	0.529	1.333	0.280	1.000	0.880	0.358		0.053	0.949	1.000	1.000	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A45: ANOVA table (3) of CW testing of mid-aged Pink1 x DAT mice: interlimb coordination and individual paw

			Pink1 x DAT, mid-aged, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
interlimb coordination	21	Number of patterns	4.264	0.019	7.349	0.009	3.453	0.038	0.128	1.000	0.015	4.111	0.027	1.000	0.103	0.036	3.114	0.059	0.055	0.544	0.709
	22	Regularity index [%]	0.724	0.489	0.515	0.476	0.618	0.542	1.000	0.743	1.000	1.157	0.329	1.000	0.966	0.438	0.385	0.684	1.000	1.000	1.000
	23	Phase dispersion [%] RF-LH	1.175	0.316	4.081	0.048	2.212	0.119	0.791	1.000	0.346	1.607	0.218	0.717	1.000	0.278	3.735	0.036	0.162	1.000	0.039
		LF-RH	1.019	0.367	0.044	0.835	0.023	0.977	0.613	1.000	0.741	0.436	0.651	1.000	1.000	1.000	0.614	0.548	1.000	1.000	0.982
		LH-RH	0.119	0.888	0.348	0.557	1.721	0.188	1.000	1.000	1.000	0.466	0.632	1.000	1.000	1.000	1.797	0.183	0.205	1.000	0.802
		LF-RF	1.108	0.337	0.413	0.523	0.471	0.627	0.424	1.000	1.000	0.695	0.508	1.000	1.000	0.892	0.915	0.411	0.782	0.725	1.000
		RF-RH	6.031	0.004	0.844	0.362	0.022	0.978	0.339	0.191	0.003	2.409	0.108	1.000	0.673	0.111	4.082	0.027	0.527	0.452	0.023
		LF-LH	4.801	0.012	0.713	0.402	1.474	0.237	0.042	1.000	0.018	2.074	0.145	1.000	0.658	0.159	4.399	0.021	0.020	1.000	0.154
	24	Couplings [%] RF-LH	0.163	0.850	0.150	0.700	0.009	0.991	1.000	1.000	1.000	0.163	0.851	1.000	1.000	1.000	0.069	0.934	1.000	1.000	1.000
		LF-RH	4.607	0.014	0.291	0.592	0.254	0.776	0.071	1.000	0.016	2.284	0.120	0.593	1.000	0.133	2.560	0.094	0.162	1.000	0.179
		LH-RF	1.358	0.265	1.553	0.218	0.349	0.707	0.515	1.000	0.521	0.758	0.478	0.839	1.000	0.969	4.920	0.014	0.088	1.000	0.015
		RH-LF	3.176	0.049	0.359	0.551	0.144	0.866	0.232	1.000	0.050	1.783	0.187	0.749	1.000	0.221	1.405	0.261	0.574	1.000	0.381
		LH-RH	0.015	0.986	0.165	0.686	0.898	0.413	1.000	1.000	1.000	0.275	0.762	1.000	1.000	1.000	0.799	0.459	0.725	1.000	0.992
		LF-RF	1.064	0.352	0.342	0.561	0.388	0.680	0.495	0.796	1.000	0.410	0.668	1.000	1.000	1.000	1.033	0.368	0.792	0.582	1.000
		RH-LH	0.030	0.971	0.213	0.646	1.601	0.210	1.000	1.000	1.000	0.497	0.614	1.000	1.000	1.000	1.282	0.292	0.482	1.000	0.550
		RF-LF	0.962	0.388	0.005	0.941	0.680	0.511	0.495	1.000	1.000	0.911	0.414	0.792	1.000	0.745	0.800	0.459	1.000	0.669	1.000
		RF-RH	6.004	0.004	0.799	0.375	0.021	0.979	0.353	0.186	0.003	2.409	0.108	1.000	0.673	0.111	4.048	0.028	0.557	0.437	0.024
		LF-LH	6.224	0.004	1.417	0.239	1.222	0.302	0.031	1.000	0.004	2.074	0.145	1.000	0.658	0.159	6.219	0.006	0.009	1.000	0.021
		RH-RF	5.610	0.006	0.811	0.372	0.034	0.967	0.372	0.224	0.004	2.549	0.096	1.000	0.664	0.096	3.167	0.057	0.715	0.593	0.053
		LH-LF	6.337	0.003	2.304	0.135	0.673	0.514	0.028	1.000	0.003	1.917	0.166	1.000	1.000	0.181	5.699	0.008	0.018	1.000	0.018
individual paw	25	% Max contact at [%] RF	1.746	0.183	11.343	0.001	1.895	0.159	1.000	0.662	0.416	2.675	0.086	0.912	0.086	0.597	1.088	0.350	0.552	1.000	0.708
		RH	0.944	0.395	0.073	0.787	0.077	0.926	1.000	0.570	0.993	0.374	0.691	1.000	1.000	1.000	0.702	0.504	1.000	0.738	1.000
		LF	1.453	0.242	3.245	0.077	0.148	0.863	1.000	0.329	0.933	0.962	0.394	1.000	0.532	1.000	0.653	0.528	1.000	0.999	1.000
		LH	0.640	0.531	0.039	0.845	0.369	0.693	1.000	0.967	1.000	0.715	0.498	1.000	0.834	1.000	0.029	0.972	1.000	1.000	1.000
		FP	2.629	0.081	11.276	0.001	1.320	0.275	1.000	0.194	0.286	2.679	0.086	0.780	0.085	0.699	1.186	0.319	0.986	1.000	0.412
		HP	0.125	0.883	0.085	0.772	0.264	0.769	1.000	1.000	1.000	0.216	0.807	1.000	1.000	1.000	0.159	0.854	1.000	1.000	1.000
		RF	1.630	0.205	20.631	0.000	0.989	0.378	0.233	0.587	1.000	2.367	0.112	0.215	0.199	1.000	0.107	0.899	1.000	1.000	1.000
		RH	0.875	0.422	22.982	0.000	0.261	0.771	0.569	0.952	1.000	0.561	0.577	1.000	0.908	1.000	0.516	0.602	0.955	1.000	1.000
		LF	1.569	0.217	18.431	0.000	0.712	0.495	0.404	0.418	1.000	2.125	0.138	0.596	0.154	1.000	0.225	0.800	1.000	1.000	1.000
		LH	0.597	0.554	22.457	0.000	0.677	0.512	0.835	1.000	1.000	0.732	0.490	1.000	0.713	1.000	0.336	0.717	1.000	1.000	1.000
		FP	1.666	0.198	20.423	0.000	0.866	0.426	0.281	0.463	1.000	2.343	0.115	0.322	0.152	1.000	0.162	0.851	1.000	1.000	1.000
		HP	0.785	0.461	24.445	0.000	0.504	0.607	0.631	1.000	1.000	0.715	0.498	1.000	0.733	1.000	0.427	0.656	1.000	1.000	1.000
	26	Max contact max intensity [pixel] RF	0.432	0.651	7.729	0.007	0.564	0.572	1.000	1.000	1.000	0.641	0.534	1.000	0.879	1.000	0.151	0.861	1.000	1.000	1.000
		RH	0.350	0.706	19.782	0.000	0.441	0.646	1.000	1.000	1.000	0.415	0.664	1.000	1.000	1.000	0.340	0.715	1.000	1.000	1.000
		LF	0.620	0.542	12.084	0.001	0.618	0.543	1.000	1.000	1.000	0.980	0.388	1.000	0.537	1.000	0.000	1.000	1.000	1.000	1.000
		LH	0.758	0.473	16.897	0.000	1.655	0.200	0.555	1.000	0.955	1.307	0.287	0.720	0.401	1.000	1.048	0.363	1.000	1.000	0.498
		FP	0.538	0.587	10.360	0.002	0.595	0.555	1.000	1.000	1.000	0.823	0.449	1.000	0.670	1.000	0.040	0.961	1.000	1.000	1.000
		HP	0.564	0.572	19.427	0.000	1.026	0.365	0.711	1.000	1.000	0.857	0.435	1.000	0.642	1.000	0.681	0.514	1.000	1.000	0.759
	27	Max contact mean intensity [pixel] RF	0.614	0.545	7.797	0.007	0.418	0.661	1.000	0.997	1.000	0.639	0.535	1.000	0.805	1.000	0.065	0.937	1.000	1.000	1.000
		RH	0.194	0.824	8.744	0.004	1.098	0.340	1.000	1.000	1.000	0.947	0.400	1.000	0.578	1.000	0.218	0.805	1.000	1.000	1.000
		LF	0.700	0.501	12.101	0.001	0.796	0.456	1.000	0.914	1.000	1.056	0.361	1.000	0.473	1.000	0.003	0.997	1.000	1.000	1.000
		LH	0.153	0.859	10.121	0.002	1.522	0.227	1.000	1.000	1.000	0.725	0.493	1.000	0.717	1.000	0.966	0.392	1.000	0.758	0.705
		FP	0.677	0.512	10.188	0.002	0.621	0.541	1.000	0.926	1.000	0.858	0.435	1.000	0.602	1.000	0.019	0.981	1.000	1.000	1.000
		HP	0.109	0.897	10.477	0.002	1.476	0.237	1.000	1.000	1.000	0.879	0.427	1.000	0.595	1.000	0.619	0.545	1.000	0.997	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A46: ANOVA table (4) of CW testing of mid-aged Pink1 x DAT mice: individual paw

			Pink1 x DAT, mid-aged, CW									males					females				
			Genotype		Sex		Interaction		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value			Genotype		Post hoc Bonferroni, p-value		
			F-value	p-value	F-value	p-value	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2	F-value	p-value	mt vs ctrl.1	mt vs ctrl.2	ctrl.1 vs ctrl.2
individual paw	28 Maximum intensity [pixel]	RF	0.518	0.599	9.720	0.003	0.491	0.614	1.000	1.000	1.000	0.679	0.515	1.000	0.813	1.000	0.050	0.951	1.000	1.000	1.000
		RH	0.272	0.763	17.955	0.000	0.339	0.714	1.000	1.000	1.000	0.300	0.743	1.000	1.000	1.000	0.293	0.748	1.000	1.000	1.000
		LF	0.506	0.606	15.659	0.000	0.900	0.412	1.000	1.000	1.000	1.024	0.372	1.000	0.491	1.000	0.040	0.961	1.000	1.000	1.000
		LH	0.684	0.509	15.833	0.000	1.283	0.285	0.618	1.000	1.000	1.005	0.379	0.896	0.562	1.000	0.915	0.411	1.000	1.000	0.569
		FP	0.536	0.588	13.167	0.001	0.696	0.503	1.000	1.000	1.000	0.868	0.431	1.000	0.608	1.000	0.018	0.982	1.000	1.000	1.000
		HP	0.493	0.613	17.851	0.000	0.808	0.451	0.799	1.000	1.000	0.652	0.529	1.000	0.855	1.000	0.617	0.547	1.000	1.000	0.829
	29 % Maximum intensity at [sec]	RF	1.289	0.283	1.140	0.290	0.043	0.958	1.000	0.429	0.558	1.082	0.353	1.000	0.643	0.625	0.426	0.657	1.000	1.000	1.000
		RH	0.461	0.633	10.316	0.002	0.054	0.947	1.000	1.000	1.000	0.151	0.860	1.000	1.000	1.000	0.368	0.695	1.000	1.000	1.000
		LF	1.322	0.274	7.218	0.009	1.202	0.308	1.000	0.877	0.212	2.083	0.143	0.186	1.000	0.457	1.087	0.350	1.000	0.503	0.936
		LH	1.089	0.343	2.087	0.154	2.051	0.138	1.000	0.585	1.000	3.129	0.059	0.676	0.056	0.592	0.087	0.917	1.000	1.000	1.000
		FP	1.456	0.242	3.778	0.057	0.319	0.728	1.000	0.487	0.270	1.054	0.362	1.000	1.000	0.483	0.876	0.427	1.000	0.644	1.000
		HP	1.075	0.348	8.400	0.005	0.629	0.537	1.000	0.563	1.000	1.566	0.227	1.000	0.263	1.000	0.067	0.935	1.000	1.000	1.000
	30 Minimum intensity [pixel]	RF	0.637	0.532	3.592	0.063	0.116	0.891	1.000	0.892	1.000	0.756	0.479	1.000	0.715	1.000	0.099	0.906	1.000	1.000	1.000
		RH	0.686	0.508	11.093	0.002	0.611	0.546	0.629	1.000	1.000	0.012	0.988	1.000	1.000	1.000	1.068	0.356	0.503	1.000	0.881
		LF	1.351	0.267	0.642	0.426	1.513	0.229	0.520	0.563	1.000	1.601	0.220	1.000	0.254	0.976	0.942	0.401	0.729	1.000	0.703
		LH	0.712	0.495	10.036	0.002	1.472	0.238	0.627	1.000	1.000	0.741	0.486	1.000	0.718	1.000	1.321	0.282	0.741	1.000	0.377
		FP	0.994	0.376	2.366	0.129	0.652	0.525	1.000	0.616	1.000	1.280	0.294	1.000	0.369	1.000	0.229	0.797	1.000	1.000	1.000
		HP	0.850	0.433	12.672	0.001	1.009	0.371	0.503	1.000	0.887	0.254	0.777	1.000	1.000	1.000	1.373	0.269	0.480	1.000	0.462
	31 Mean intensity mean [pixel]	RF	0.838	0.438	4.984	0.029	0.448	0.641	1.000	0.717	1.000	0.795	0.461	1.000	0.663	1.000	0.158	0.855	1.000	1.000	1.000
		RH	0.307	0.737	8.818	0.004	0.743	0.480	1.000	1.000	1.000	0.724	0.493	1.000	0.820	1.000	0.072	0.931	1.000	1.000	1.000
		LF	0.582	0.562	8.833	0.004	0.982	0.381	1.000	1.000	1.000	1.147	0.332	1.000	0.430	1.000	0.061	0.940	1.000	1.000	1.000
		LH	0.136	0.873	9.109	0.004	0.921	0.404	1.000	1.000	1.000	0.587	0.563	1.000	0.898	1.000	0.374	0.691	1.000	1.000	1.000
		FP	0.736	0.483	7.053	0.010	0.722	0.490	1.000	0.849	1.000	0.989	0.385	1.000	0.520	1.000	0.064	0.938	1.000	1.000	1.000
		HP	0.202	0.818	9.765	0.003	0.928	0.401	1.000	1.000	1.000	0.689	0.510	1.000	0.814	1.000	0.243	0.786	1.000	1.000	1.000
	32 Intensity of 15 most intense pixel [pixel]	RF	0.881	0.420	8.199	0.006	0.373	0.690	1.000	0.713	1.000	0.825	0.449	1.000	0.629	1.000	0.169	0.845	1.000	1.000	1.000
		RH	0.378	0.687	16.551	0.000	0.504	0.606	1.000	1.000	1.000	0.542	0.588	1.000	0.926	1.000	0.250	0.781	1.000	1.000	1.000
		LF	0.618	0.542	13.401	0.001	0.612	0.546	1.000	1.000	1.000	0.954	0.397	1.000	0.535	1.000	0.024	0.976	1.000	1.000	1.000
		LH	0.411	0.665	15.360	0.000	1.104	0.339	0.937	1.000	1.000	0.794	0.462	1.000	0.679	1.000	0.666	0.521	1.000	1.000	0.799
		FP	0.782	0.462	11.040	0.002	0.513	0.601	1.000	0.823	1.000	0.929	0.407	1.000	0.551	1.000	0.077	0.926	1.000	1.000	1.000
		HP	0.408	0.667	16.850	0.000	0.848	0.434	0.951	1.000	1.000	0.710	0.500	1.000	0.746	1.000	0.467	0.632	1.000	1.000	1.000
	33 Print length [cm]	RF	1.741	0.184	6.371	0.014	0.169	0.845	0.778	0.223	1.000	1.471	0.247	0.598	0.350	1.000	0.656	0.526	1.000	0.831	1.000
		RH	0.386	0.681	10.880	0.002	0.209	0.812	1.000	1.000	1.000	0.819	0.451	0.755	0.917	1.000	0.031	0.969	1.000	1.000	1.000
		LF	0.857	0.430	12.882	0.001	0.149	0.862	1.000	0.689	1.000	1.044	0.365	1.000	0.491	1.000	0.142	0.868	1.000	1.000	1.000
		LH	0.794	0.457	17.025	0.000	0.525	0.595	0.506	1.000	1.000	1.128	0.338	0.604	0.609	1.000	0.317	0.731	1.000	1.000	1.000
		FP	1.458	0.241	9.949	0.003	0.146	0.865	0.889	0.324	1.000	1.477	0.246	0.716	0.318	1.000	0.395	0.677	1.000	1.000	1.000
		HP	0.666	0.518	16.050	0.000	0.386	0.682	0.616	1.000	1.000	1.137	0.335	0.566	0.642	1.000	0.158	0.854	1.000	1.000	1.000
	34 Print Width [cm]	RF	1.428	0.248	9.911	0.003	0.605	0.549	0.563	0.426	1.000	2.387	0.110	0.537	0.118	1.000	0.135	0.874	1.000	1.000	1.000
		RH	1.882	0.161	17.206	0.000	0.693	0.504	0.141	0.754	1.000	1.748	0.193	0.438	0.284	1.000	0.863	0.432	0.700	1.000	0.881
		LF	1.834	0.169	6.140	0.016	0.448	0.641	0.921	0.201	1.000	1.282	0.293	1.000	0.502	0.549	1.048	0.363	0.759	0.585	1.000
		LH	0.206	0.815	25.477	0.000	0.461	0.633	1.000	1.000	1.000	0.549	0.584	1.000	0.919	1.000	0.152	0.859	1.000	1.000	1.000
		FP	1.826	0.170	9.081	0.004	0.335	0.717	0.628	0.232	1.000	1.935	0.163	1.000	0.183	0.718	0.503	0.610	1.000	1.000	1.000
		HP	0.844	0.435	23.193	0.000	0.635	0.533	0.499	1.000	1.000	1.124	0.339	0.969	0.458	1.000	0.429	0.655	1.000	1.000	1.000
	35 Print area [cm]	RF	1.664	0.198	13.363	0.001	0.473	0.626	0.574	0.304	1.000	2.163	0.134	0.560	0.150	1.000	0.173	0.842	1.000	1.000	1.000
		RH	0.945	0.395	20.428	0.000	0.156	0.856	0.427	1.000	1.000	0.589	0.562	1.000	1.000	1.000	0.469	0.630	1.000	1.000	1.000
		LF	1.467	0.239	14.087	0.000	0.439	0.647	0.623	0.379	1.000	1.905	0.168	1.000	0.184	0.889	0.303	0.741	1.000	1.000	1.000
		LH	0.556	0.576	23.046	0.000	0.657	0.522	0.776	1.000	1.000	0.732	0.490	1.000	0.732	1.000	0.338	0.716	1.000	1.000	1.000
		FP	1.663	0.199	14.353	0.000	0.454	0.637	0.561	0.308	1.000	2.203	0.129	0.713	0.137	1.000	0.224	0.800	1.000	1.000	1.000
		HP	0.804	0.452	23.641	0.000	0.422	0.658	0.520	1.000	1.000	0.720	0.496	1.000	0.789	1.000	0.408	0.669	1.000	1.000	1.000

red: p-value ≤ 0.05, yellow = p-value ≥ 0.05 ≤ 0.1, RF = right front paws, RH = right hind paws, LF = left front paws, LH = left hind paws, FP = front paws, HP = hind paws. Table XY summarises the description of the CW parameters.

Table A47: Summary of results of selected CW parameters of young and mid-aged Pink1 x Pet mice

Pink1 x Pet

Minimum intensity [pixel] of LF paws

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	29.19	0.47	females	ctrl. 1	14	29.10	0.49	males	ctrl. 1	12	14.50	0.13	females	ctrl. 1	12	14.54	0.20
	mt	9	28.74	0.38		mt	10	29.11	0.40		mt	11	14.47	0.18		mt	10	14.54	0.17
	ctrl. 2	9	29.28	0.39		ctrl. 2	14	28.92	0.58		ctrl. 2	9	14.51	0.21		ctrl. 2	13	14.49	0.30

% Max contact at [%] of LF paws

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	37.73	7.75	females	ctrl. 1	14	32.81	7.08	males	ctrl. 1	12	38.61	7.06	females	ctrl. 1	12	34.85	7.39
	mt	9	35.00	7.91		mt	10	40.02	4.93		mt	11	32.71	6.72		mt	10	42.37	3.81
	ctrl. 2	9	37.19	7.59		ctrl. 2	14	33.24	5.92		ctrl. 2	9	31.98	5.81		ctrl. 2	13	36.02	5.44

% Max contact at [%] of FP

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	41.85	4.27	females	ctrl. 1	14	37.94	6.16	males	ctrl. 1	12	41.45	6.25	females	ctrl. 1	12	40.69	5.00
	mt	9	40.79	6.38		mt	10	42.08	3.89		mt	11	37.81	5.58		mt	10	45.66	4.02
	ctrl. 2	9	40.33	6.51		ctrl. 2	14	38.14	4.98		ctrl. 2	9	36.80	4.08		ctrl. 2	13	38.57	4.85

Phase dispersion [%] of LF-RF paws

young										mid-aged									
age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD	age	groups	n	mean	SD
males	ctrl. 1	12	51.08	1.83	females	ctrl. 1	14	49.49	1.65	males	ctrl. 1	12	51.18	2.46	females	ctrl. 1	12	49.28	2.26
	mt	9	50.39	1.66		mt	10	50.96	2.14		mt	11	50.61	1.74		mt	10	52.77	2.57
	ctrl. 2	9	50.85	1.45		ctrl. 2	14	50.24	2.35		ctrl. 2	9	50.81	2.11		ctrl. 2	13	49.67	1.68

LF = left front paws, FP = front paws, RF = right front paws

Table A48: Summary (1) of results of selected CW parameters of young and mid-aged Pink1 x DAT mice

Pink1 x DAT

Stance duration [sec] of RH paws

young										mid-aged									
age	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**
males	ctrl. 1	12	0.10	0.01	females	ctrl. 1	10	0.11	0.02	males	ctrl. 1	11	0.10	0.01	females	ctrl. 1	10	0.12	0.02
	mt	11	0.11	0.01		mt	12	0.12	0.02		mt	10	0.11	0.01		mt	11	0.12	0.02
	ctrl. 2	12	0.09	0.01		ctrl. 2	12	0.11	0.02		ctrl. 2	10	0.11	0.01		ctrl. 2	12	0.13	0.02

Stance duration [sec] of HP

young										mid-aged									
age	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**
males	ctrl. 1	12	0.10	0.01	females	ctrl. 1	10	0.11	0.02	males	ctrl. 1	11	0.10	0.01	females	ctrl. 1	10	0.12	0.02
	mt	11	0.11	0.01		mt	12	0.12	0.01		mt	10	0.11	0.01		mt	11	0.12	0.02
	ctrl. 2	12	0.10	0.01		ctrl. 2	12	0.11	0.01		ctrl. 2	10	0.11	0.01		ctrl. 2	12	0.13	0.01

Body speed [cm/sec] of LH paws

young										mid-aged									
age	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**
males	ctrl. 1	12	27.96	3.97	females	ctrl. 1	10	27.06	5.50	males	ctrl. 1	11	20.79	3.77	females	ctrl. 1	10	20.21	3.64
	mt	11	25.39	4.95		mt	12	23.18	4.12		mt	10	20.78	2.91		mt	11	21.49	5.91
	ctrl. 2	12	29.44	5.88		ctrl. 2	12	25.59	3.97		ctrl. 2	10	23.78	5.01		ctrl. 2	12	21.30	3.10

Body speed [cm/sec] of HP

young										mid-aged									
age	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**	**age**	**groups**	**n**	**mean**	**SD**
males	ctrl. 1	12	27.87	3.92	females	ctrl. 1	10	26.88	5.38	males	ctrl. 1	11	20.83	3.93	females	ctrl. 1	10	20.15	3.77
	mt	11	25.38	4.91		mt	12	23.25	4.17		mt	10	20.83	2.84		mt	11	21.40	5.96
	ctrl. 2	12	29.41	5.99		ctrl. 2	12	25.59	3.97		ctrl. 2	10	23.64	5.00		ctrl. 2	12	21.27	3.08

RH = right hind paws, HP = hind paws, LH = left hind paws

Table A49: Summary (2) of results of selected CW parameters of young and mid-aged Pink1 x DAT mice

Pink1 x DAT

Swing speed [cm/sec] of LH paws

young

age	groups	n	mean	SD
males	ctrl. 1	12	69.01	9.29
	mt	11	61.80	9.65
	ctrl. 2	12	72.25	8.99

age	groups	n	mean	SD
female	ctrl. 1	10	58.41	8.90
	mt	12	50.51	8.05
	ctrl. 2	12	56.22	7.80

mid-aged

age	groups	n	mean	SD
males	ctrl. 1	11	61.85	8.35
	mt	10	53.95	8.49
	ctrl. 2	10	61.34	7.51

age	groups	n	mean	SD
females	ctrl. 1	10	54.51	9.92
	mt	11	48.94	7.28
	ctrl. 2	12	50.68	7.84

Swing speed [cm/sec] of HP

young

age	groups	n	mean	SD
males	ctrl. 1	12	66.85	7.65
	mt	11	61.47	9.63
	ctrl. 2	12	69.42	9.90

age	groups	n	mean	SD
females	ctrl. 1	10	57.74	7.16
	mt	12	50.23	8.06
	ctrl. 2	12	55.09	7.08

mid-aged

age	groups	n	mean	SD
males	ctrl. 1	11	60.67	8.39
	mt	10	52.89	7.56
	ctrl. 2	10	59.64	9.60

age	groups	n	mean	SD
females	ctrl. 1	10	52.03	7.89
	mt	11	49.50	8.09
	ctrl. 2	12	50.22	6.62

Base of support [cm] of HP

young

age	groups	n	mean	SD
males	ctrl. 1	12	2.90	0.23
	mt	11	2.65	0.18
	ctrl. 2	12	2.80	0.16

age	groups	n	mean	SD
females	ctrl. 1	10	2.61	0.20
	mt	12	2.70	0.18
	ctrl. 2	12	2.71	0.17

mid-aged

age	groups	n	mean	SD
males	ctrl. 1	11	3.19	0.22
	mt	10	2.81	0.21
	ctrl. 2	10	3.07	0.20

age	groups	n	mean	SD
females	ctrl. 1	10	2.85	0.23
	mt	11	2.91	0.16
	ctrl. 2	12	2.88	0.15

LH = left hind paws, HP = hind paws

Table A50: ANOVA table oflfac. testing of young and mid-aged males Pink1 x Pet and Pink1 x DAT mice

males Pink1 x Pet, young, Olfac.

		Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	fl,+ vs. wt,+	fl,+ vs fl,-	wt,+ vs fl,-
binary mixtures	100 % [S+]	0.284	0.756	1.000	1.000	1.000
	70 % [S+]	0.347	0.711	1.000	1.000	1.000
	55 % [S+]	0.319	0.731	1.000	1.000	1.000
	53 % [S+]	1.018	0.380	.580	.934	1.000
	51 % [S+]	0.679	0.519	.972	1.000	.966
sensitivity	threshold dilution steps of [S+]	0.476	0.629	1.000	1.000	1.000

males Pink1 x Pet, mid-aged, Olfac.

		Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	fl,+ vs. wt,+	fl,+ vs fl,-	wt,+ vs fl,-
binary mixtures	100 % [S+]	0.260	0.774	1.000	1.000	1.000
	70 % [S+]	4.332	0.029	0.028	1.000	0.274
	55 % [S+]	0.516	0.605	0.970	1.000	1.000
	53 % [S+]	0.332	0.722	1.000	1.000	1.000
	51 % [S+]	0.794	0.467	0.768	1.000	1.000
sensitivity	threshold dilution steps of [S+]	0.223	0.802	1.000	1.000	1.000

males Pink1 x DAT, young, Olfac.

		Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	fl,+ vs. wt,+	fl,+ vs fl,-	wt,+ vs fl,-
binary mixtures	100 % [S+]	0.787	0.469	0.830	0.881	1.000
	70 % [S+]	2.022	0.159	0.241	1.000	0.379
	55 % [S+]	0.017	0.983	1.000	1.000	1.000
	53 % [S+]	0.041	0.960	1.000	1.000	1.000
	51 % [S+]	0.698	0.509	1.000	0.767	1.000
sensitivity	threshold dilution steps of [S+]	1.448	0.259	0.323	1.000	0.950

males Pink1 x DAT, mid-aged, Olfac.

		Genotype		post-hoc Bonferoni p-value		
		F-value	p-value	fl,+ vs. wt,+	fl,+ vs fl,-	wt,+ vs fl,-
binary mixtures	100 % [S+]	0.998	0.390	1.000	1.000	0.565
	70 % [S+]	1.543	0.244	0.343	1.000	0.604
	55 % [S+]	0.987	0.394	1.000	0.563	1.000
	53 % [S+]	1.351	0.287	0.362	1.000	1.000
	51 % [S+]	0.353	0.708	1.000	1.000	1.000
sensitivity	threshold dilution steps of [S+]	0.493	0.620	1.000	1.000	1.000

Table A51: Summary of results of olfactory binary mixture and sensitivity testing of young and mid-aged Pink1 x Pet and Pink1 x DAT mice

		males Pink1 x Pet, young								
		ctrl.1			mt			ctrl.2		
		mean	SD	n	mean	SD	N	mean	SD	n
binary mixtures	100 % of [S+]	83.76	14.09	7	87.79	9.21	8	87.64	10.98	7
	70 % of [S+]	86.96	12.23	7	87.46	11.41	8	82.09	16.64	7
	55 % of [S+]	73.81	21.21	7	72.92	19.80	8	66.67	12.73	7
	53 % of [S+]	66.67	15.96	7	56.25	8.63	8	64.29	19.07	7
	51 % of [S+]	72.62	17.82	7	64.58	13.91	8	64.29	14.20	7
sensitivity	threshold of dilution steps of [S+]	12.86	2.85	7	13.25	3.62	8	11.71	2.75	7

		males Pink1 x Pet, mid-aged								
		ctrl.1			mt			ctrl.2		
		mean	SD	n	mean	SD	n	mean	SD	n
binary mixtures	100 % of [S+]	96.43	6.56	7	96.88	4.31	8	94.44	8.61	6
	70 % of [S+]	95.24	4.45	7	75.00	12.60	8	81.94	20.01	6
	55 % of [S+]	82.86	12.79	7	73.96	20.62	8	77.78	15.52	6
	53 % of [S+]	60.71	18.46	7	64.58	17.11	8	69.44	22.77	6
	51 % of [S+]	58.33	10.76	7	66.67	17.82	8	59.72	9.74	6
sensitivity	threshold of dilution steps of [S+]	13.29	7.54	7	12.63	7.50	8	10.67	6.59	6

		males Pink1 x DAT, young								
		ctrl.1			mt			ctrl.2		
		mean	SD	n	mean	SD	n	mean	SD	n
binary mixtures	100 % of [S+]	94.70	11.76	8	89.34	8.74	7	94.51	6.37	8
	70 % of [S+]	96.88	6.20	8	86.90	10.60	7	88.54	13.32	8
	55 % of [S+]	83.33	14.09	8	82.14	18.28	7	82.29	8.26	8
	53 % of [S+]	77.08	18.77	8	75.00	16.67	7	77.08	12.40	8
	51 % of [S+]	82.29	18.06	8	78.57	13.49	7	87.50	11.79	8
sensitivity	threshold of dilution steps of [S+]	7.63	1.60	8	13.14	8.91	7	10.88	6.62	8

		males Pink1 x DAT, mid-aged								
		ctrl.1			mt			ctrl.2		
		mean	SD	n	mean	SD	n	mean	SD	n
binary mixtures	100 % of [S+]	97.22	6.80	6	93.06	6.27	6	91.67	8.33	7
	70 % of [S+]	69.44	31.91	6	87.50	6.97	6	83.33	6.80	7
	55 % of [S+]	69.44	14.59	6	73.61	13.35	6	61.90	17.25	7
	53 % of [S+]	61.11	16.39	6	73.61	14.35	6	66.67	8.33	7
	51 % of [S+]	52.78	11.39	6	58.33	15.81	6	52.38	14.20	7
sensitivity	threshold of dilution steps of [S+]	12.83	7.11	6	11.33	5.85	6	14.86	6.28	7

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Affidavit

Eidesstattliche Versicherung/Affidavit

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation „Analysis of a murine Pink1 deficiency in serotonergic and dopaminergic neurons in respect of Parkinson´s disease“ selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

I hereby confirm that the dissertation “Analysis of a murine Pink1 deficiency in serotonergic and dopaminergic neurons in respect of Parkinson´s disease” is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

München, den 09.06.2018 Unterschrift Angelika Hummel
Munich, date signature

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Declaration of author contribution

The animal care takers of the C-Streifen of the HMGU, especially Vanessa Sill, took care of the mice by checking the health status in a daily manner. They refreshed the animal boxes and refilled fresh water and food.

Susanne Badeke, a technician of the Institute of Developmental Genetics (IDG) of the HMGU contributed to the genotyping process of the different mouse lines.

Dr. Florian Giesert, Postdoc and Group leader of the (IDG) of the HMGU, supported me during the preparation of mouse brains for immunohistochemically studies, neurotransmitter analysis and mouse line characterization.

The following cutting of the mouse brains were mainly done by the technician Susanne Badeke. The internship students Theresa Baumeister and Katharina Bach contributed partially the cutting of mouse brains. The subsequent immunohistochemical staining were done by Susanne Badeke and partially contributed by the students Theresa Baumeister.

Additionally, Susanne Badeke quantified the 5-HT^+ fibres in selected Pink1 x Pet and Pink1 x DAT mice. The polymerase reaction of selected brain regions of the Pink1 x Pet mice for the characterization of the mouse line was also performed by Susanne Badeke.

After purifying the brain samples on my own, samples were handed over to the PhD student Joachim Nagel of the Intitute Melecular EXposomics (MEX) of the HMGU. He performed the HPLC measurements and analysis of the HPLC peaks of the young Pink1 x Pet and Pink1 x DAT mice. Dr. Meri De Angelis from the MEX of the HMGU performed the HPLC measurements and analysis of the mid-aged Pink1 x Pet and Pink1 DAT cohorts and analysed the HPLC peaks.

The technician of the IDG, Bettina Sperling, performed the open field testing of the mid-aged Pink1 x Pet mice. In addition, she shared the acoustic startle reflex/prepulse inhibition testing with the technician Jan Einicke of the mid-aged Pink1 x Pet mice. The olfactory testing of the young and mid-aged Pink1 x Pet and Pink1 x DAT mice including analysis was performed by Bettina Sperling.